MRC/DBT Workshop UK-India Centre for Advanced Technologies-Minimising the indiscriminate use of Antibiotics (UKICAT–MA)

14th/15th March 2016, Hyderabad, India

Preface

On 14/15th March 2016 we held a MRC/DBT funded workshop on the theme of Materials to Combat Antibiotic Resistance. The workshop was part of a continuing series of events that are part of the work of UK-India Centre for Advanced Technologies-Minimising the indiscriminate use of Antibiotics (UKICAT–MA). The following is the collection of presentations and the results of discussions highlighting key themes for future work by this group.

Combating antibiotic resistance is perhaps the biggest issue facing the global community in the 21st century and no other area, with the exception perhaps of nuclear conflict, has the capacity to significantly reduce living standards and mortality rates. Key objectives identified by WHO[1] in this area among five key aspects, include:

Objective 4-to optimize the use of antimicrobial agents
Objective 5-new medicines, diagnostic tools, vaccines and other interventions

Our aims in this series of workshops are to provide an Indo-UK forum for: discussions of our advances in providing technologies to address these objectives; facilitate the interface between UK and Indian clinicians, materials and biological scientists and to identify key areas for new projects. An important aspect of the work in a global context is that by combining the UK and Indian community and clinical experiences we cover most of the scenarios that the global population might expect to encounter.

Our first workshop focused on bringing together materials experts, microbiologists and clinical practitioners and scientists. On clinical aspects Prashant Garg and Ian Douglas both provided presentations that set the scene and discussed the environment for antibiotic resistance that is causing such great concern. Savitri Sharma, emphasized the role that fungal infections play in tropical climates.

Subhadeep Chatterjee provided key insights into the role of quorum sensing and pointed out some of the key molecules that might provide useful technological targets.

A wide range of useful technologies were presented at the workshop. In terms of emerging technology we heard about a number of novel delivery systems from Vamsi Krishna Vanugentu, Pradip Paik and Saptarshi Majumdar. Vamsi Krishna Vanugentu described the use of electrical charge to promote diffusion of nanoparticles towards the infective tissues. Pradip Paik discussed a several nanoparticle delivery systems and showed how molecular imprinting might be used to selectively bind important biomolecules and pointed out the importance of chirality. Saptarshil Majumber discussed their work on delivery from hydrogels, fibres as well as coarse grain modeling of polymer behaviour in aqueous media. Joey Shepherd showed us how ultra-sound could be used to break up biofilms. This MRC/DBT project is mainly funded to further our work on branched polymers that are responsive to bacteria and Stephen Rimmer provided an update of our progress in this area and showed how responsive polymers can be used to target different organisms.

1 WHO. Global Action Plan on Anti-microbial Resistance. (2015).

"UK-India Centre for Advanced Technology for Minimising the indiscriminate use of Antibiotics (UKICAT-MA)

Workshop (March 14-15, 2016)

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Bacterial Resistance: A Growing Concern

Prashant Garg; MD
L V Prasad Eye Institute, Hyderabad

Antibiotic Resistance

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So that all may see

Objective

- Phenomenon of drug resistance
- State of affairs for systemic infections
- State of affairs for ocular infections
- Global initiative
- Lessons we can learn

Antibiotic Resistance

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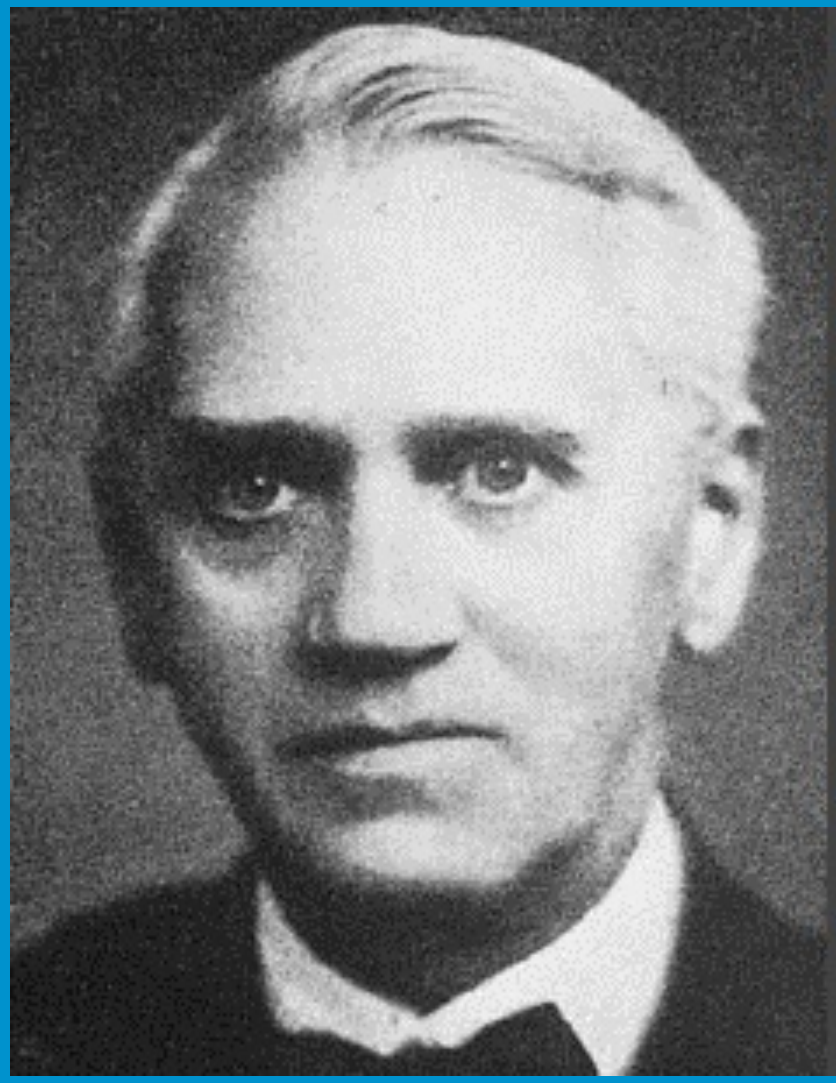
Antibiotics are expected
to become
less effective over time

Pathogens continue to evolve

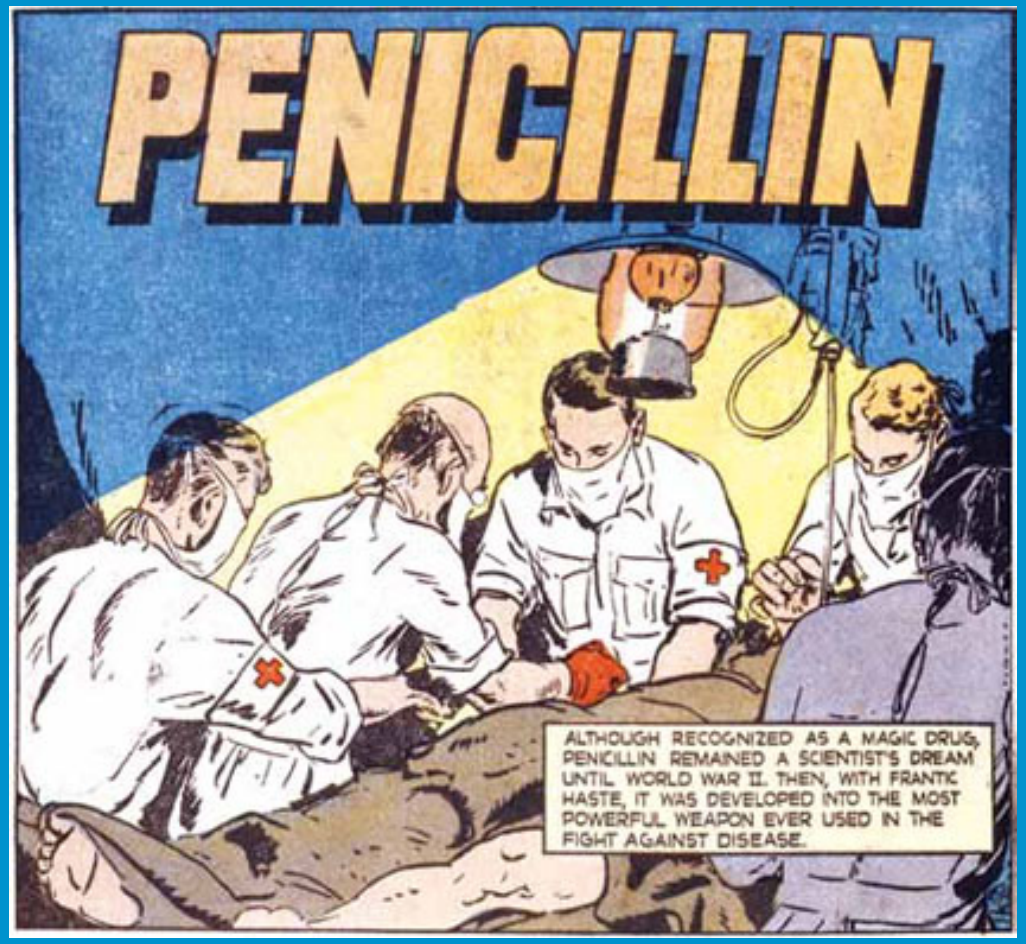
Antibiotic



"It has been demonstrated that a species of penicillium produces in culture a very powerful antibacterial substance which affects different bacteria in different degrees. Generally speaking it may be said that the least sensitive bacteria are the Gram-negative bacilli, and the most susceptible are the pyogenic cocci ... In addition to its possible use in the treatment of bacterial infections penicillin is certainly useful... for its power of inhibiting unwanted microbes in bacterial cultures so that penicillin insensitive bacteria can readily be isolated."

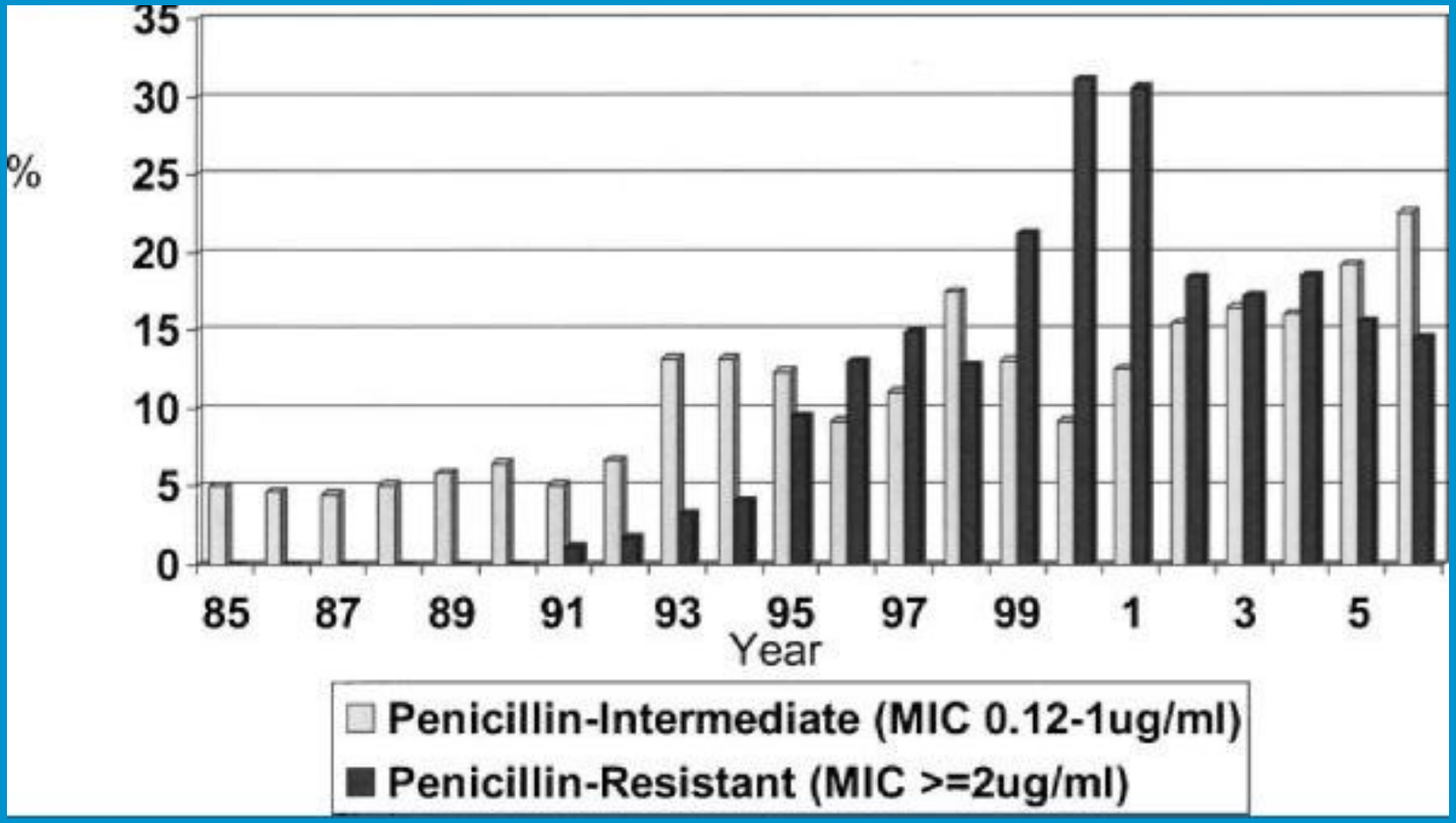
Alexander Fleming

Antibiotic

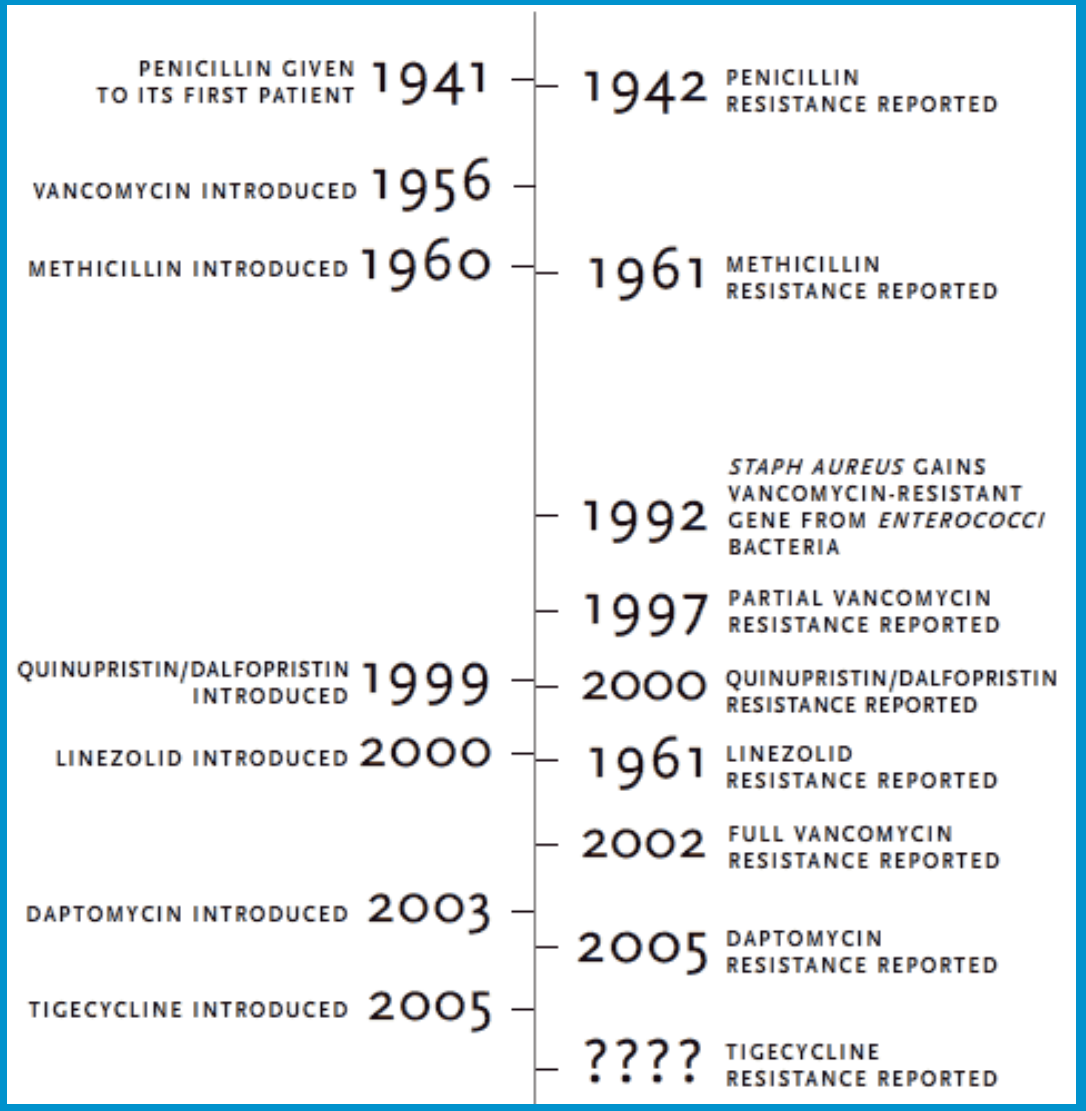

PENICILLIN
ALTHOUGH RECOGNIZED AS A MAGIC DRUG, PENICILLIN REMAINED A SCIENTIST'S DREAM UNTIL WORLD WAR II. THEN, WITH FRANTIC HASTE, IT WAS DEVELOPED INTO THE MOST POWERFUL WEAPON EVER USED IN THE FIGHT AGAINST DISEASE.


BUY BONDS
PENICILLIN
CURES
GONORRHEA
IN 4 HOURS
SEE YOUR DOCTOR TODAY

Antibiotic

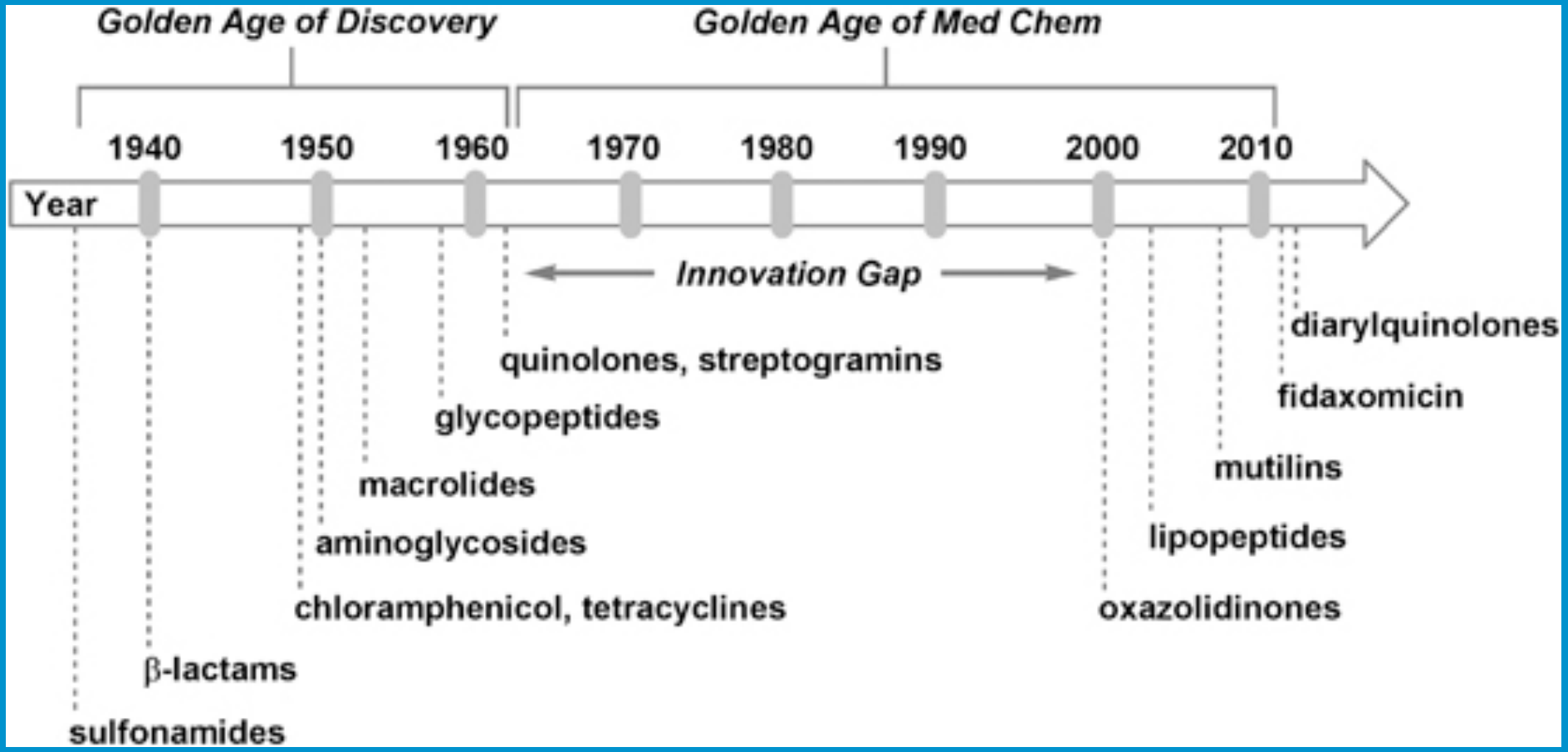

%
35
30
25
20
15
10
5
0
85
87
89
91
93
95
97
99
1
3
5
Year
Penicillin-Intermediate (MIC 0.12-1ug/ml)
Penicillin-Resistant (MIC >=2ug/ml)

Antibiotic


PENICILLIN GIVEN TO ITS FIRST PATIENT 1941
1942 PENICILLIN RESISTANCE REPORTED
VANCOMYCIN INTRODUCED 1956
METHICILLIN INTRODUCED 1960
1961 METHICILLIN RESISTANCE REPORTED
1992 STAPH AUREUS GAINS VANCOMYCIN-RESISTANT GENE FROM ENTEROCOCCI BACTERIA
1997 PARTIAL VANCOMYCIN RESISTANCE REPORTED
QUINUPRISTIN/DALFOPRISTIN INTRODUCED 1999
2000 QUINUPRISTIN/DALFOPRISTIN RESISTANCE REPORTED
LINEZOLID INTRODUCED 2000
1961 LINEZOLID RESISTANCE REPORTED
2002 FULL VANCOMYCIN RESISTANCE REPORTED
DAPTOMYCIN INTRODUCED 2003
2005 DAPTOMYCIN RESISTANCE REPORTED
TIGECYCLINE INTRODUCED 2005
???? TIGECYCLINE RESISTANCE REPORTED

Antibiotic Resistance

Challenges



Antibiotic Resistance

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Challenges

- Serious public health concern for diseases such as Malaria & Tuberculosis
- Increasingly being reported for common bacterial diseases
- Multi-drug resistance
- No more a “Hospital acquired phenomenon”

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Challenge

Infections

- Difficult to manage
- Associated with increased morbidity & mortality
- Increased duration of hospital stay
- Increased health care cost

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WHO Initiative

- 1998 adopted a resolution urging member states to take necessary actions
- 2001 published "*WHO global strategy on containment of antimicrobial resistance*"
- Surveillance of AMR as an essential part of the action plan

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Surveillance

Important objectives

- Trends of pathogens and AMR & regional variations
- Relationship between antibiotic use & resistance
- Assessment of outcomes interventions

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WHO Initiative

AMR surveillance

- IDSR in Africa
- ReLAVRA in Canada
- ARMed in Mediterranean
- EARS-net for European union
- WHONET

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Regional Initiatives

AMR surveillance

- NHSN
- PROTEKT
- SENTRY
- EARSS
- SMART, TSN, TRUST

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WHO Initiative

What are the important observations of these surveillance systems?

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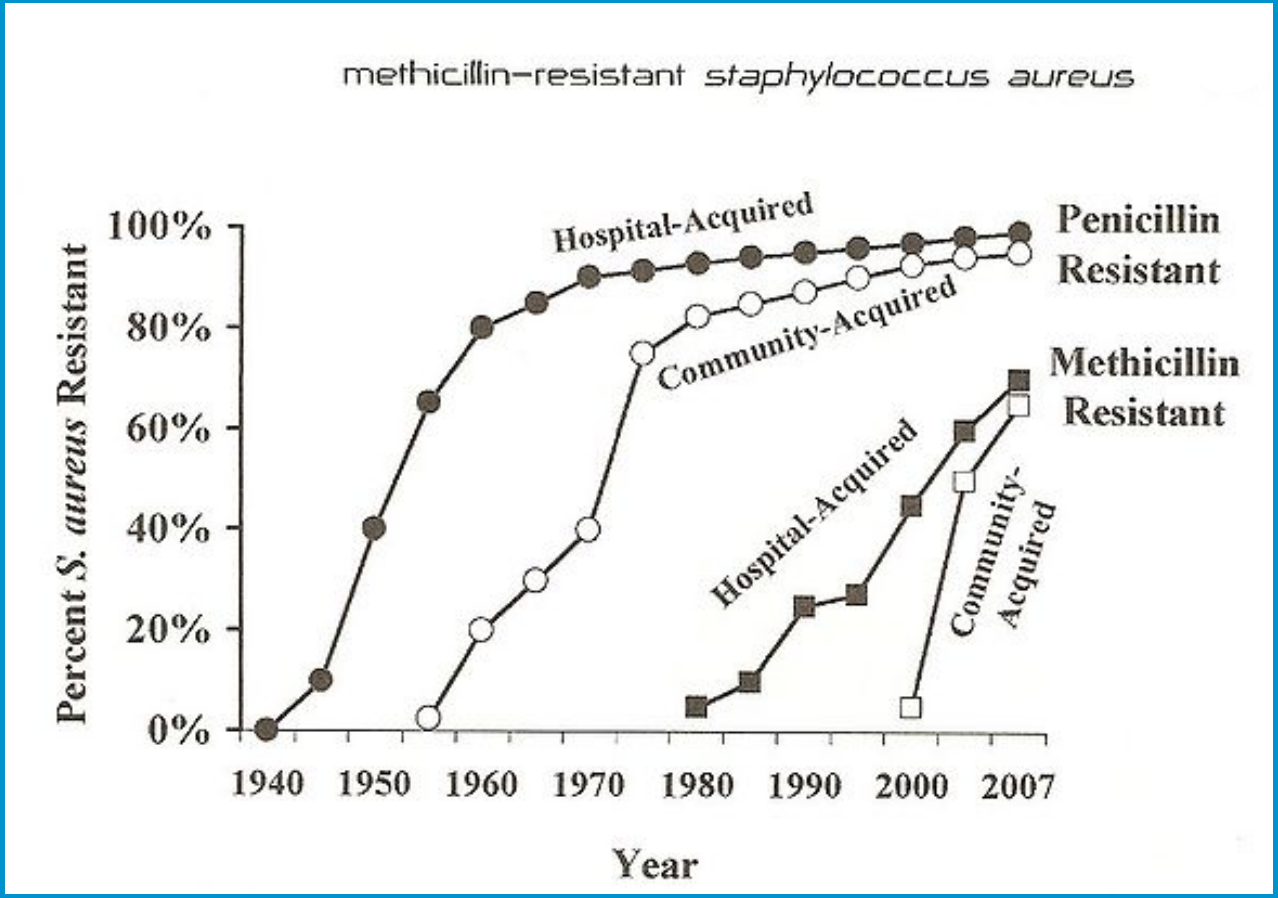
Surveillance

Important Observations

- There is a clear association between use of antibiotics & AMR.
- Time required for the development of resistance is much shorter as compared to decay after cessation.

Antibiotic Resistance



Oxacillin resistance for *S.aureus* increased but is stabilizing.

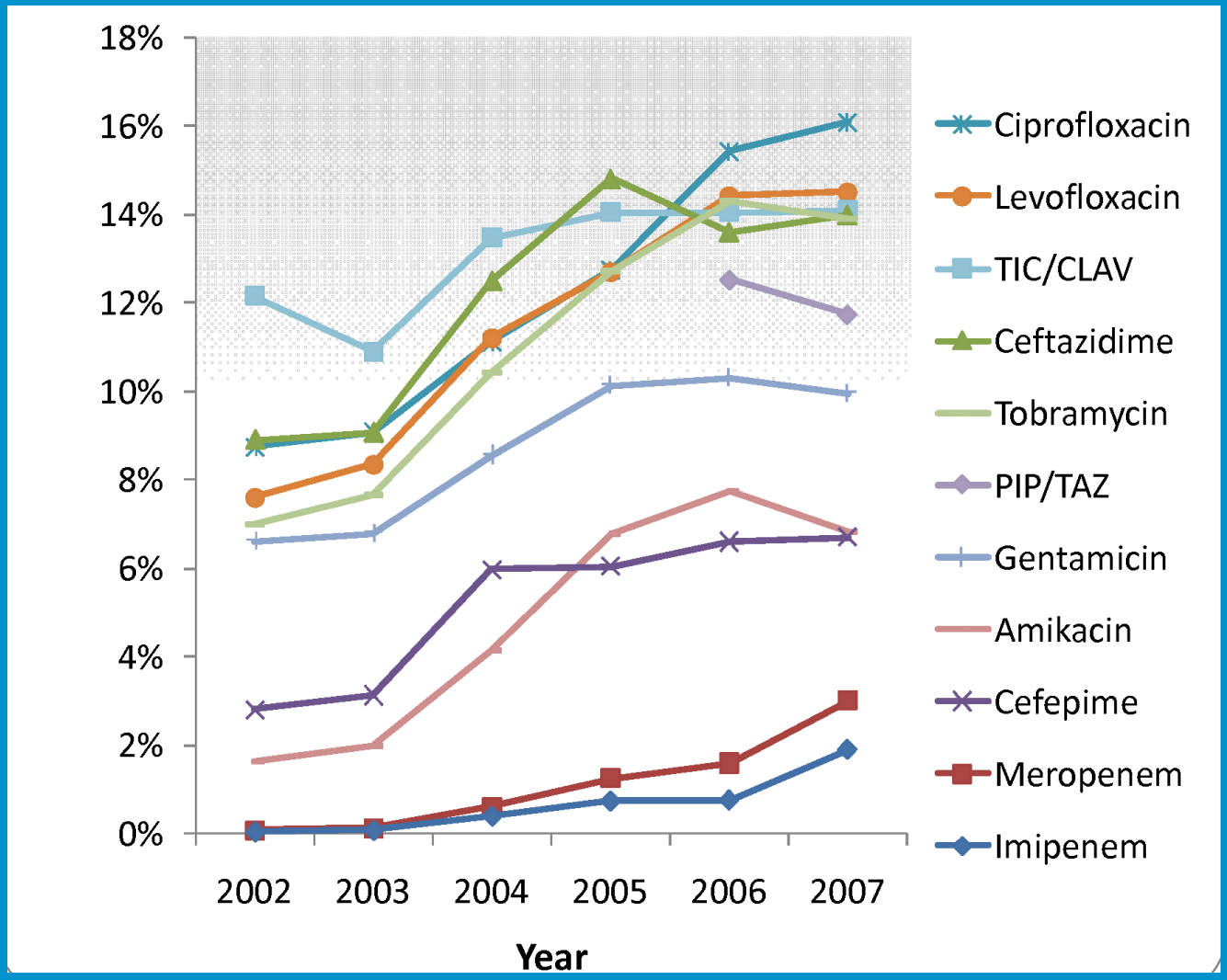
Antibiotic Resistance

Surveillance

Important Observations

- **Oxacillin resistance for *S.aureus* increased but is stabilizing.**
- **There is general increase in resistance among gram negative pathogens.**

Antibiotic Resistance



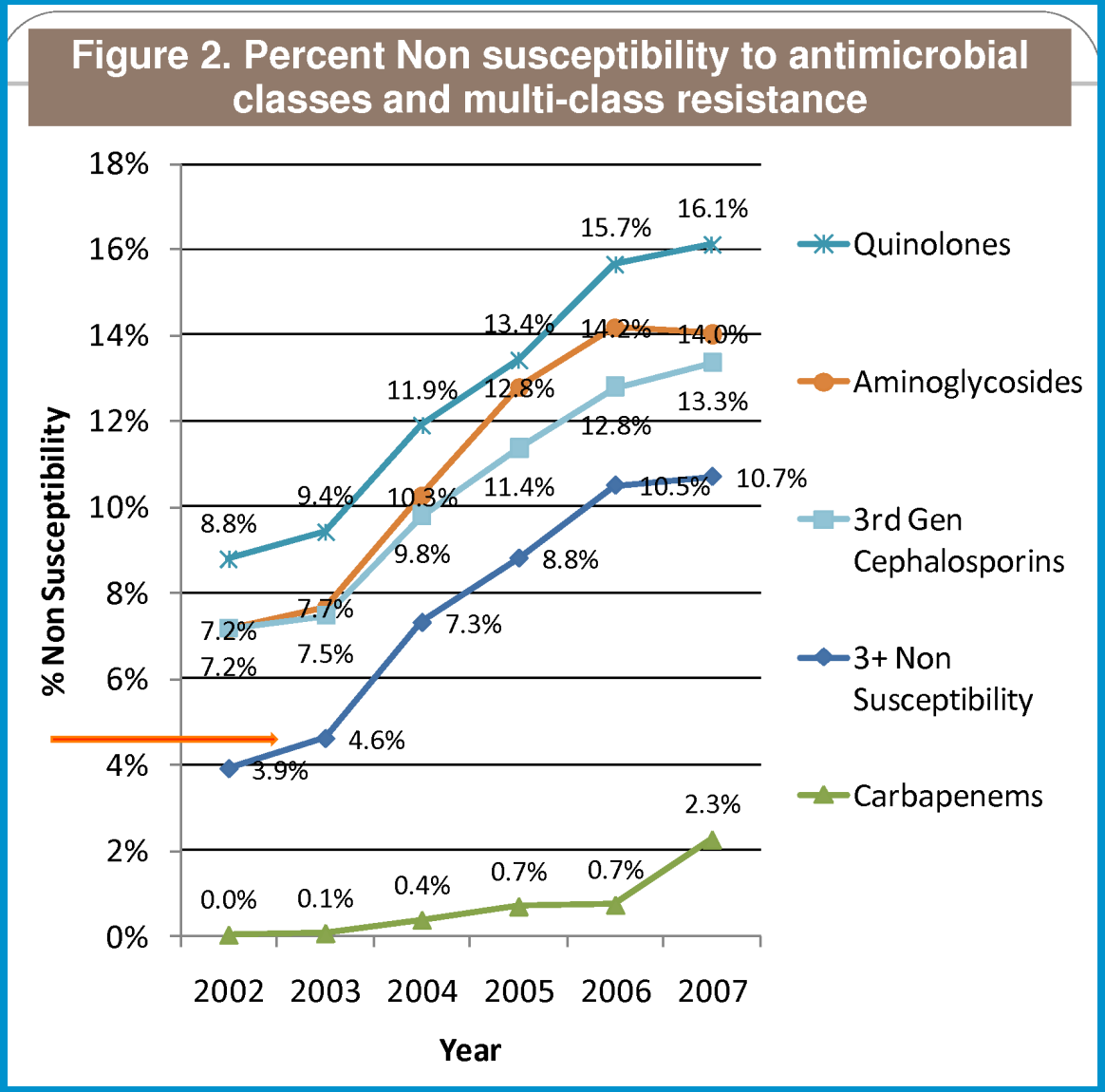
Antibiotic Resistance

Surveillance

Important Observations

- **Oxacillin resistance for *S.aureus* increased but is stabilizing.**
- **There is general increase in resistance among gram negative pathogens.**
- **There is increasing trends toward combined resistance**

Antibiotic Resistance



Antibiotic Resistance

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Surveillance

Important Observations

- Resistance among E. coli for ciprofloxacin increased from 3% in 2000 to 17.1% in 2010
- Resistance among *Acinetobacter to* imipenem increased from 23.9% to 34.3%.
- Resistance to piperacillin-tazobactam increased from 37% to 49.7%.

Antibiotic Resistance

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What is the status of resistance among ocular isolates?

Antibiotic Resistance

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Ophthalmology

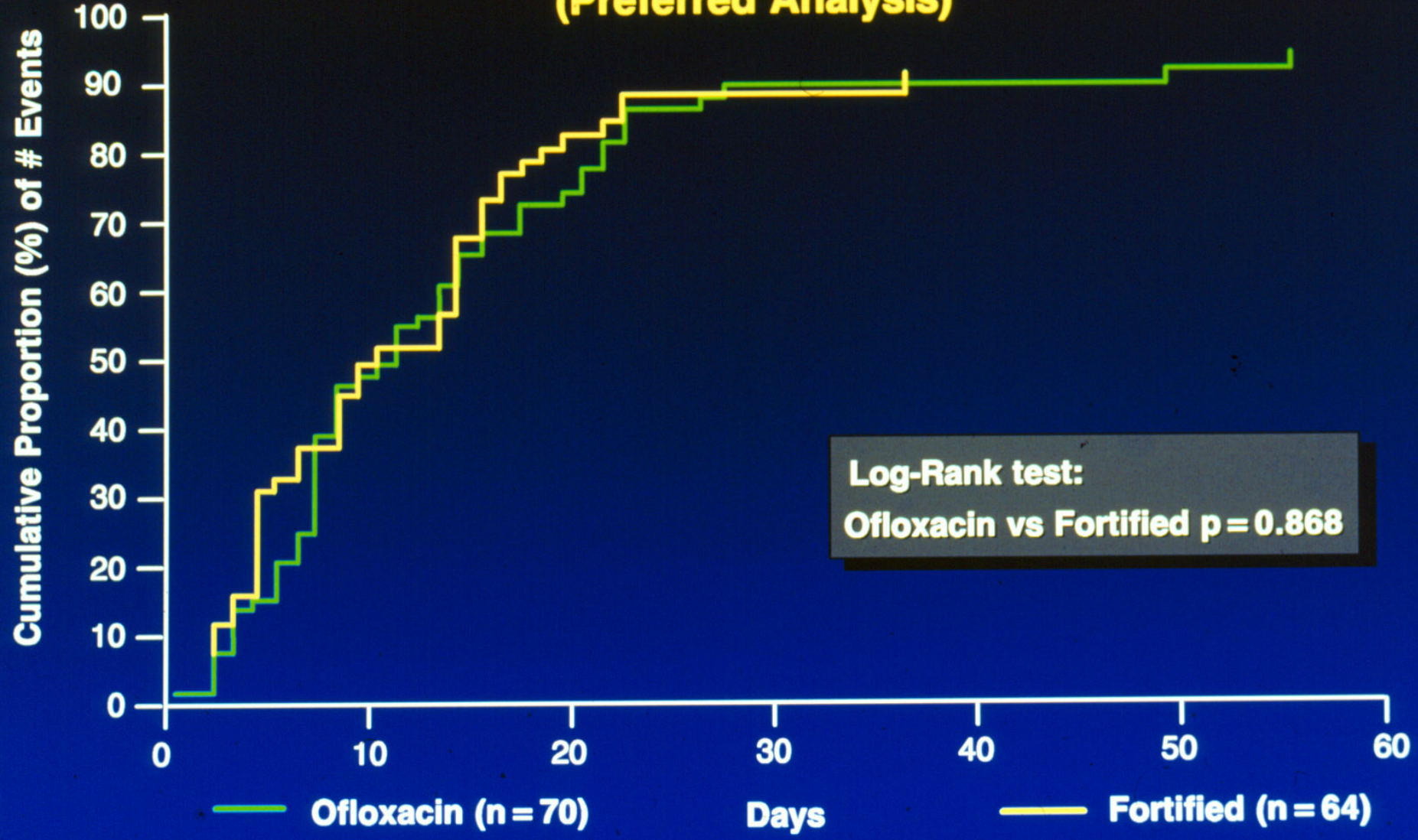
BacKerS

Bacterial Keratitis Study

Time to Clinical Success

Kaplan-Meier Estimate of Life-Table Curves

(Preferred Analysis)



Antibiotic Resistance

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Fluoroquinolones

Initial Euphoria

- Wide spread use in treatment of conjunctivitis, keratitis
- Systemic therapy
- Prophylaxis against postoperative infection

Antibiotic Resistance

Fluoroquinolones

In Vitro Susceptibility of Bacterial Keratitis Pathogens to Ciprofloxacin

Emerging Resistance

Derek Y. Kunimoto, LLB, Savitri Sharma, MD, Prashant Garg, MS, Gullapalli N. Rao, MD

Emerging Fluoroquinolone Resistance in Bacterial Keratitis

A 5-Year Review

Michael H. Goldstein, MD, Regis P. Kowalski, MS, Y. Jerold Gordon, MD

An In Vitro Resistance Study of Levofloxacin, Ciprofloxacin, and Ofloxacin Using Keratitis Isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Regis P. Kowalski, MS, [MJASCP,[1] Angana N. Pandya, MD,[1] Lisa M. Karenchak, BS, [MJASCP,[1] Eric G. Romanowski, MS,[1] Roger C. Husted, MD,[1] David C. Ritterband, MD,[2] M. K. Shah, MS,[2] Y. Jerold Gordon, MD[1]

Antibiotic Resistance

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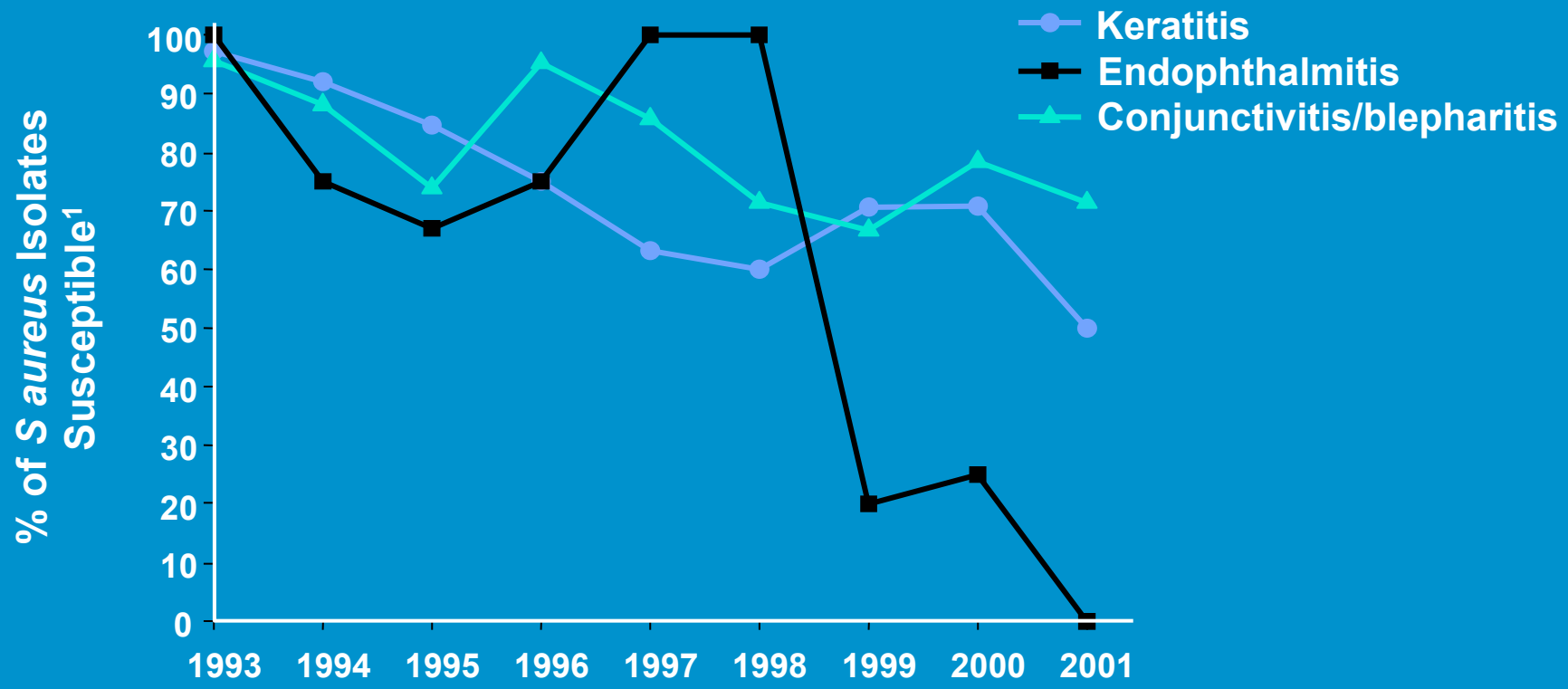
Fluoroquinolones



1. Mah F. *Ophthalmol Clin North Am.* 2003;16:11-27; 2. Marangon FB, et al. Annual Meeting of the Association for Research in Vision and Ophthalmology; May 5, 2002; Ft. Lauderdale, Fla. Abstract. 3. Ritterband DC, et al. Annual Meeting of the Association for Research in Vision and Ophthalmology; May 6, 2002; Ft. Lauderdale, Fla. Abstract.

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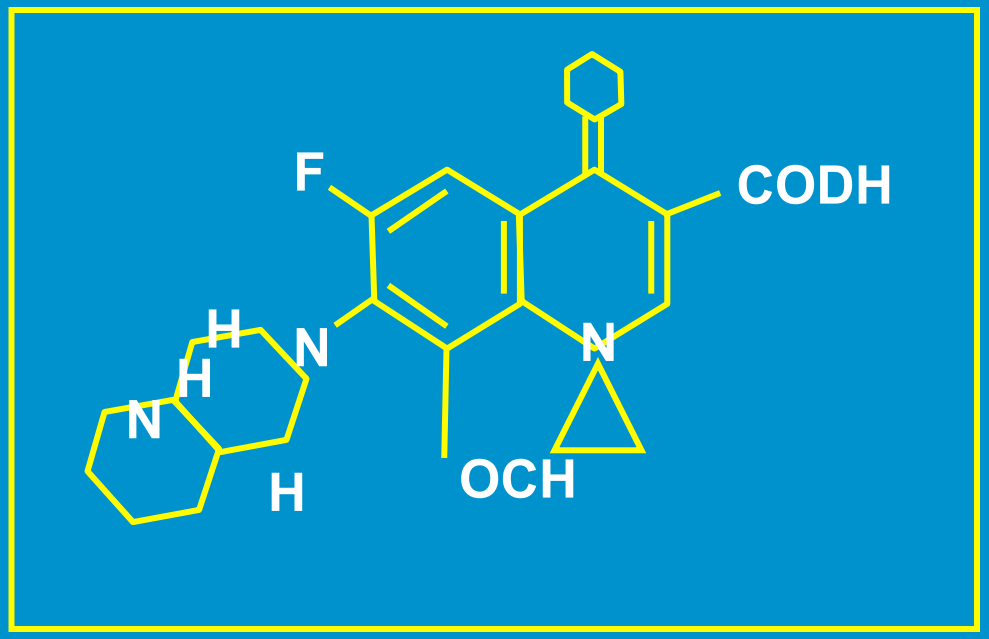
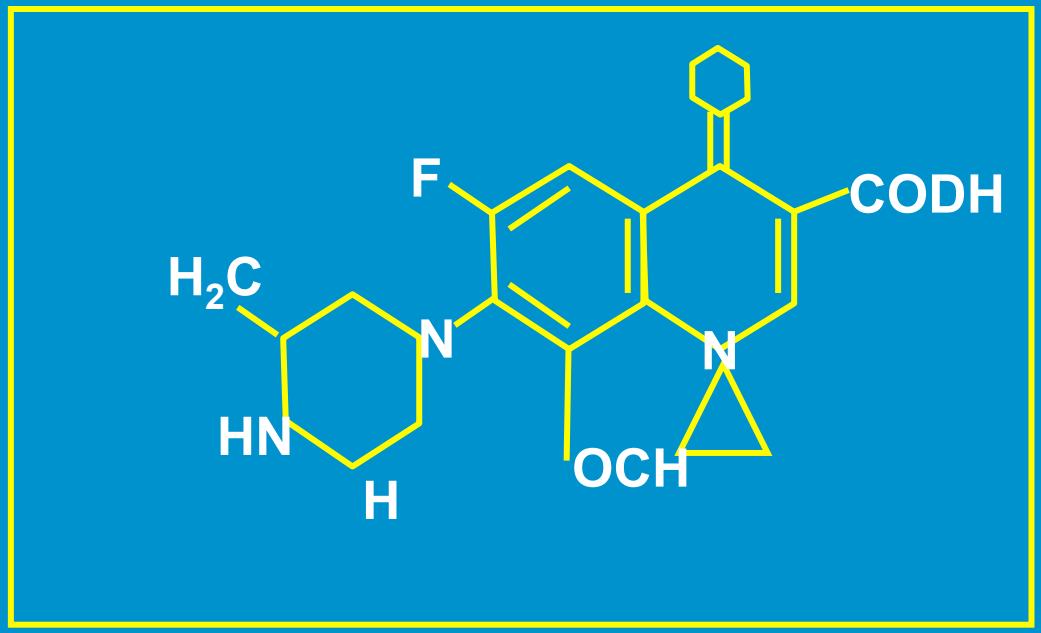
Fluoroquinolones



Moxifloxacin



Gatifloxacin

8-methoxy-6-fluoroquinolone

Antibiotic Resistance

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8-methoxy fluoroquinolones

Advantages

- Broad spectrum activity
- Superior activity against quinolone resistant isolates
- Superior kill kinetics
- Improved eradication of bacteria

Antibiotic Resistance

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Fluoroquinolones

	Ciprofloxacin	Gatifloxacin	Moxifloxacin
Cipro sensitive *Gram* + ive	0.3 (0.08-1)	0.09 (0.03-0.5)	0.09 (0.03-0.5)
Cipro resistant Gram + ive	32 (4-32)	1.5 (0-32)	1.75 (0.2-32)
Cipro sensitive *Gram* - ive	0.19 (0.13-0.75)	0.5 (0.05-3)	2 (0.13-8)
Cipro resistant *Gram* - ive	32 (32-32)	32 (32-32)	32 (32-32)

MIC in 2002

Antibiotic Resistance

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Fluoroquinolones

	Ciprofloxacin	Gatifloxacin	Moxifloxacin
Cipro sensitive *Gram +ive*	0.3 (0.08-1)	0.09 (0.03-0.5)	0.09 (0.03-0.5)
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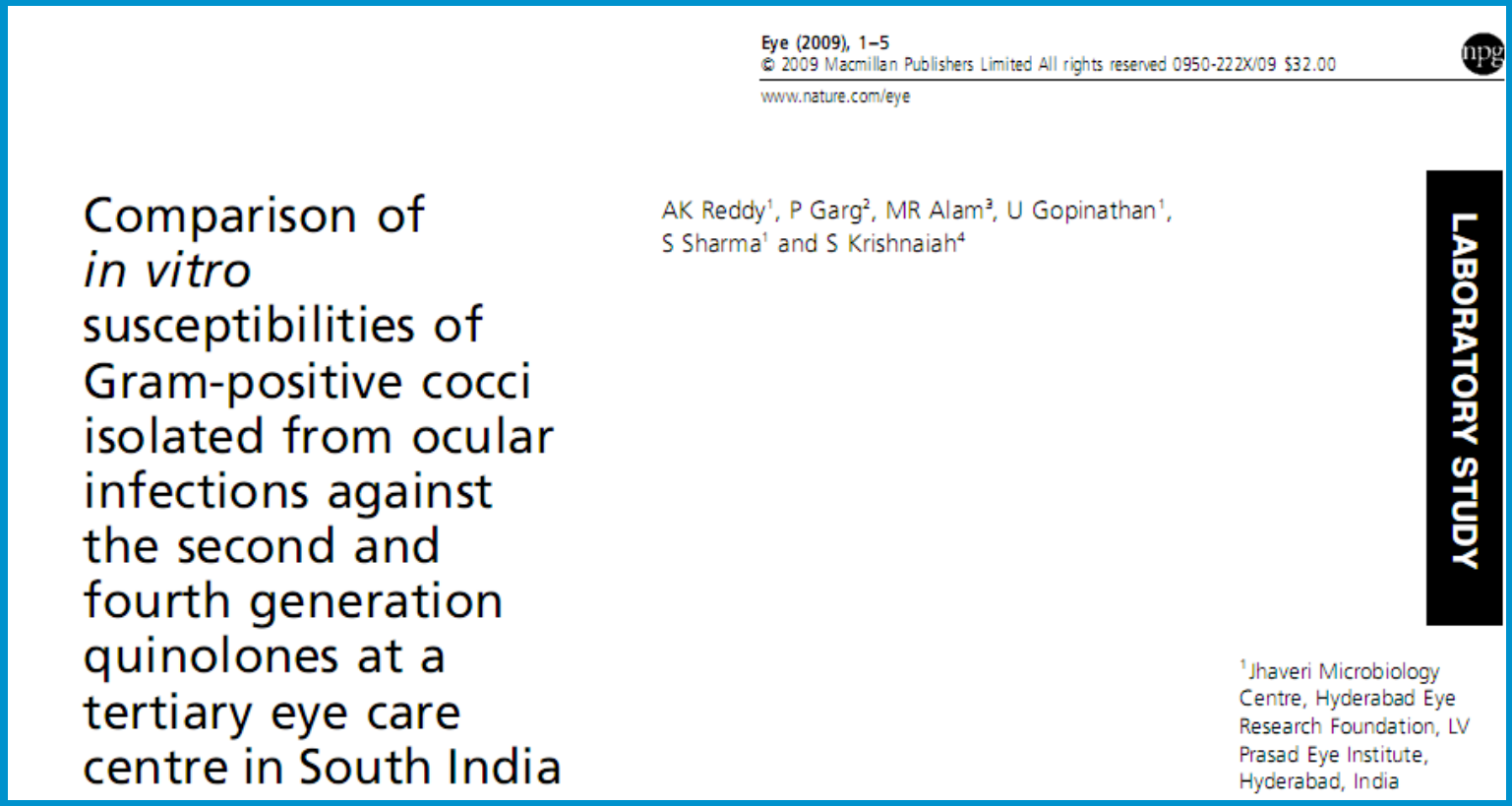
MIC in 2002

Antibiotic Resistance

Fluoroquinolones



Eye (2009), 1–5

www.nature.com/eye

Comparison of *in vitro* susceptibilities of Gram-positive cocci isolated from ocular infections against the second and fourth generation quinolones at a tertiary eye care centre in South India

AK Reddy[1], P Garg[2], MR Alam[3], U Gopinathan[1], S Sharma[1] and S Krishnaiah[4]

LABORATORY STUDY

[1]Jhaveri Microbiology Centre, Hyderabad Eye Research Foundation, LV Prasad Eye Institute, Hyderabad, India

Antibiotic Resistance

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Fluoroquinolones

Organism	Susceptibility		
	N	Gatifloxacin sensitivity (%)	Moxifloxacin sensitivity (%)
Cipro sensitive *S.aureus*	39	39 (100)	37 (94.9)
Cipro resistant *S.aureus*	97	40(41.2)	7 (7.2)
Cipro sensitive *CONS*	120	120 (100)	103 (85.3)
Cipro resistant CONS	111	84 (75.7)	28 (25.2)
Cipro sensitive *S.pneumoniae*	301	294 (97.7)	251 (83.5)
Cipro resistant *S.pneumoniae*	5	2 (40)	1 (20)

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Pseudomonas

	Susceptibility %			
Antibiotic	2009	2010	2011	2012
Amikacin	71.64	50.44	40.13	35.96
Gentamicin	64.18	47.79	40.13	32.46
Ceftazidime	38.81	37.17	37.66	33.33
Ciprofloxacin	50.71	48.67	37.66	32.46
Gatifloxacin	62.69	48.67	37.66	35.09
Moxifloxacin	50.75	42.48	35.81	29.82

Antibiotic Resistance

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Pseudomonas

	Susceptibility %			
Antibiotic	2009	2010	2011	2012
Amikacin	71.64	50.44	40.13	35.96
Gentamicin	64.18	47.79	40.13	32.46
Ceftazidime	38.81	37.17	37.66	33.33
Ciprofloxacin	50.71	48.67	37.66	32.46
Gatifloxacin	62.69	48.67	37.66	35.09
Moxifloxacin	50.75	42.48	35.81	29.82

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Ophthalmology

- Very well known in Ophthalmology & to Ophthalmologists.
- Most literature on resistance came from individual laboratories.
- Until last decade we did not have national or regional surveillance

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Ophthalmology

AMR surveillance

- **TSN: 2000 – 2005 S aureus**
- **Ocular TRUST study: *S aureus, S pneumoniae*, & *H influenzae***
- **ARMOR 2009: *S aureus*, coagulase-negative Staphylococci, *S pneumoniae*, *H influenzae* & *P. aeruginosa***

Antibiotic Resistance

Ophthalmology

AMR surveillance

- **Asia Cornea Society Infectious Keratitis Study**

ACSIKS

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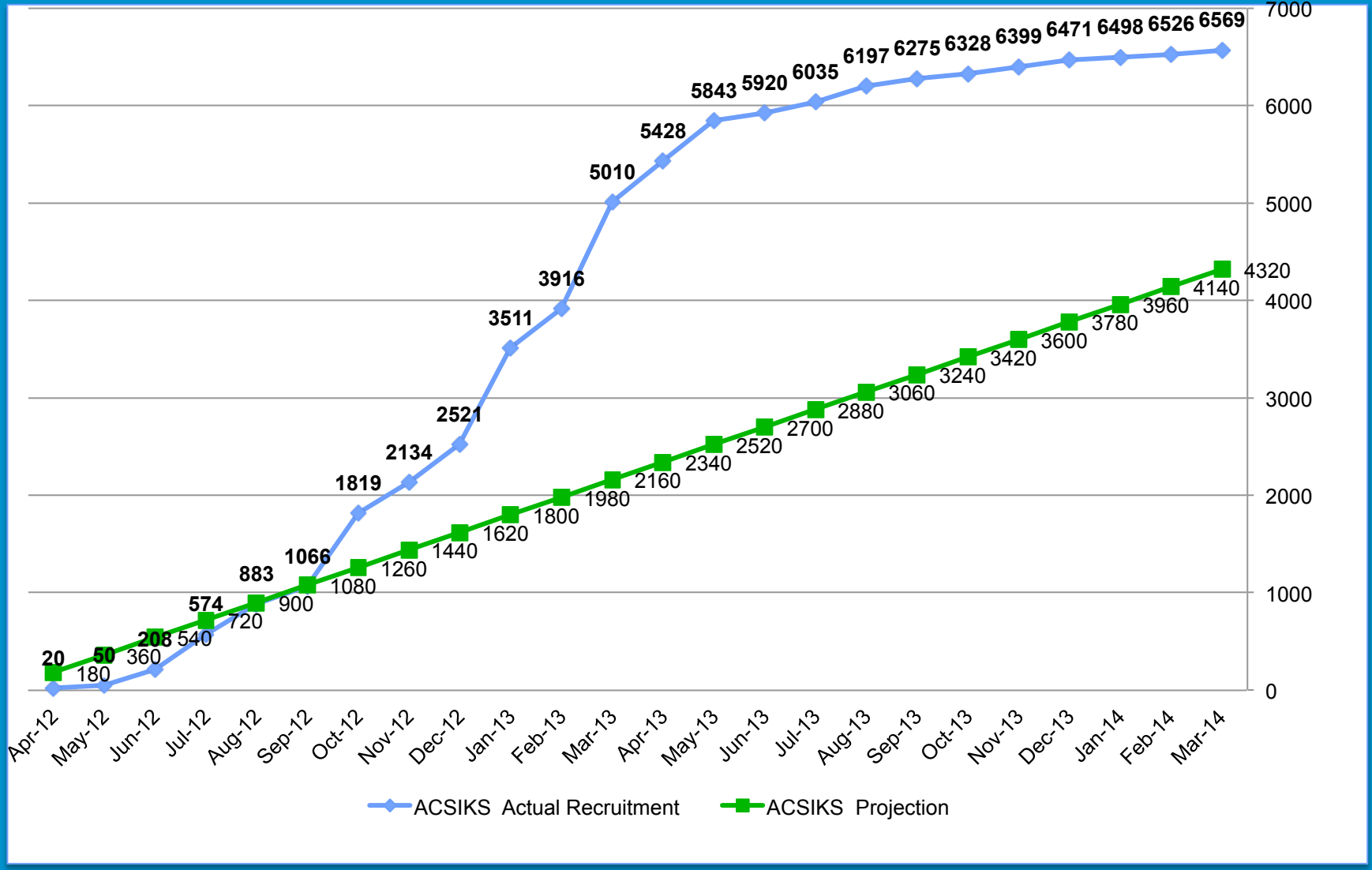
ACSIKS Study

8 Countries	12 Study Centres	27 Participating Institutions
China	Shandong Eye Institute	• Qingdao Eye Hospital • Shandong Eye Hospitals
	Xiamen Eye Centre	
India	Aravind Eye Hospitals	
	LV Prasad Eye Institute	
Japan	Tottori University Faulty of Medicine	• Eguchi Eye Hospital, Hokkaido • Miyata Eye Hospital, Kyusu
	Osaka University Graduate School of Medicine Hospital	• Kansei Rousai Hospital • Otemae Hospital • Ideta Eye Hospital • Okamoto Eye Clinic
	Kyoto Prefectural University	• Kim's Eye Hospital • Bundang Seoul National University Hospital
Korea	Seoul St Mary Hospital	• East Avenue Medical Center
Philippines	Philippine General Hospital	
Taiwan	National Taiwan University Hospital	
Thailand	Siriraj Hospital Mahidol University	• KK Women's and Children's Hospital • Changi General Hospital • National University Hospital • Tan Tock Seng Hospital • Khoo Teck Puat Hospital
Singapore	Singapore National Eye Centre	

ACSIKS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Jan-14
Feb-14
Mar-14
ACSIKS Actual Recruitment
ACSIKS Projection

ACSIKS

Enrollment Status by Country

SITE NO.	SITE	Actual Recruitment
111	Qingdao Eye Hospital (QEH)	178
112	Shandong Eye Hospital (SHA)	250
120	Xiamen Eye Centre (XEC)	512
China Total		**940**
210	Aravind Eye Hospital (AEH)	2323
220	LV Prasad Eye Institute (LVPEI)	1358
India Total		**3681**
310	Tottori University Hospital (TUH)	58
311	Eguichi Eye Hospital (EEH)	6
312	Miyata Eye Hospital (MEH)	91
320	Osaka University Grad School of Med Hospital (OUH)	54
321	Kansei Rousai Hospital (KRH)	8
322	Otemae Hospital (OH)	15
323	Ideta Eye Hospital (IEH)	20
324	Okamoto Eye Clinic (OEC)	21
910	Kyoto Prefectural University of Medicine (KPUM)	28
Japan Total		**301**

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LVPEI
So that all may see

Enrollment Status by Country

SITE NO.	SITE	Actual Recruitment
410	Seoul St Mary Hospital (SSMH)	130
411	Kim's Eye Hospital (KEH)	98
412	Bundang Seoul National University Hospital (BSNUH)	22
Korea Total		**250**
510	Philippine General Hospital (PGH)	190
511	East Ave Medical Center (EAMC)	161
Philippines Total		**351**
610	National Taiwan University Hospital (NTUH)	223
611	Mackay Memorial Hospital (MMH)	7
612	Taipei Tzu Chi General Hospital (TCGH)	3
Taiwan Total		**233**
710	Siriraj Hospital Mahidol University (SRH)	225
711	King Chulalongkorn Memorial Hospital (KCMH)	55
Thailand Total		**280**
810	Singapore National Eye Centre (SNEC)	235
812	KK Women's & Children's Hospital (KKH)	2
813	Changi General Hospital (CGH)	174
814	National University Hospital (NUH)	33
815	Tan Tock Seng Hospital (TTSH)	55
816	Khoo Teck Puat Hospital (KTPH)	34
Singapore Total		**533**

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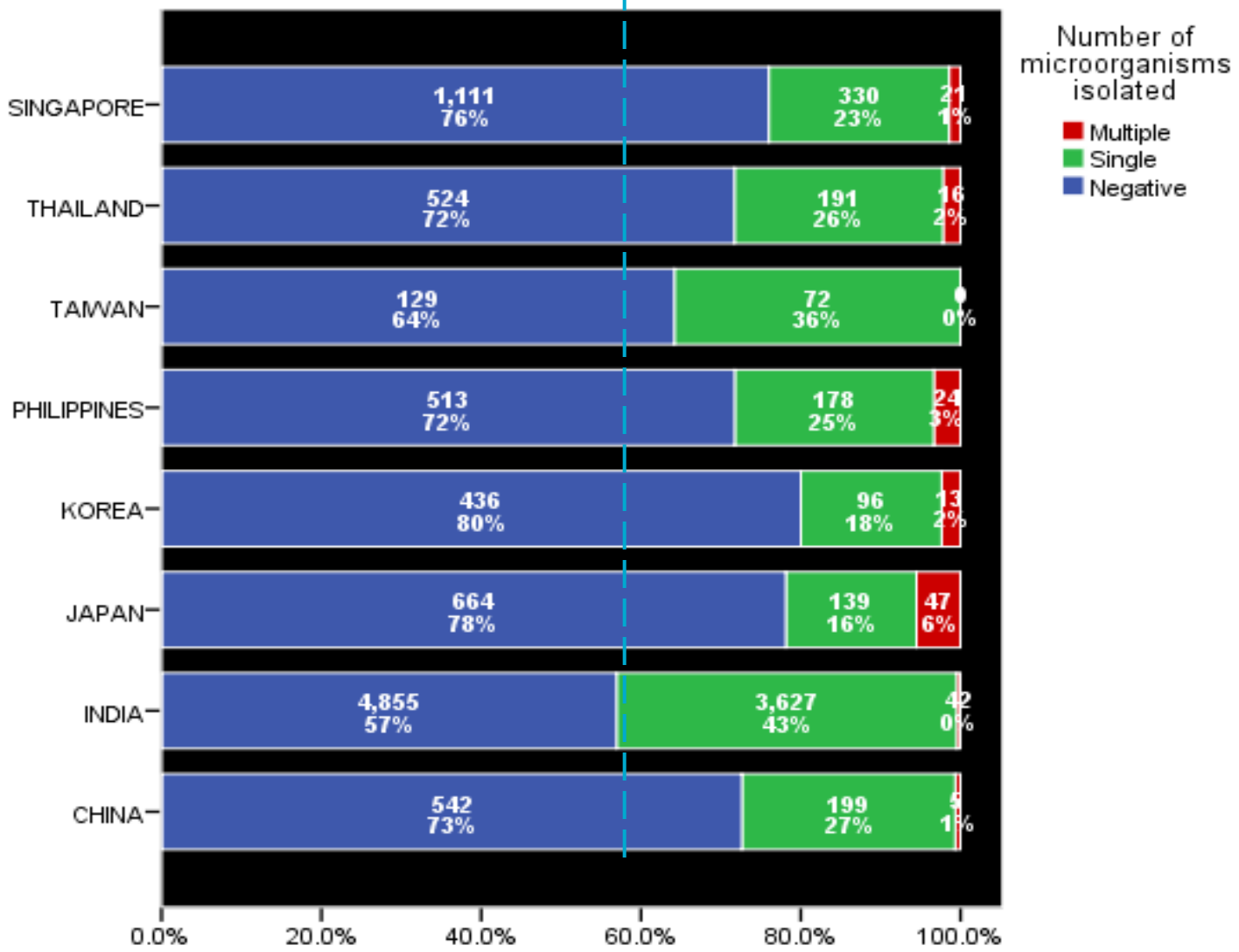
Culture Positive Rate by Country



*Includes all cultures performed; 5000 positive cultures

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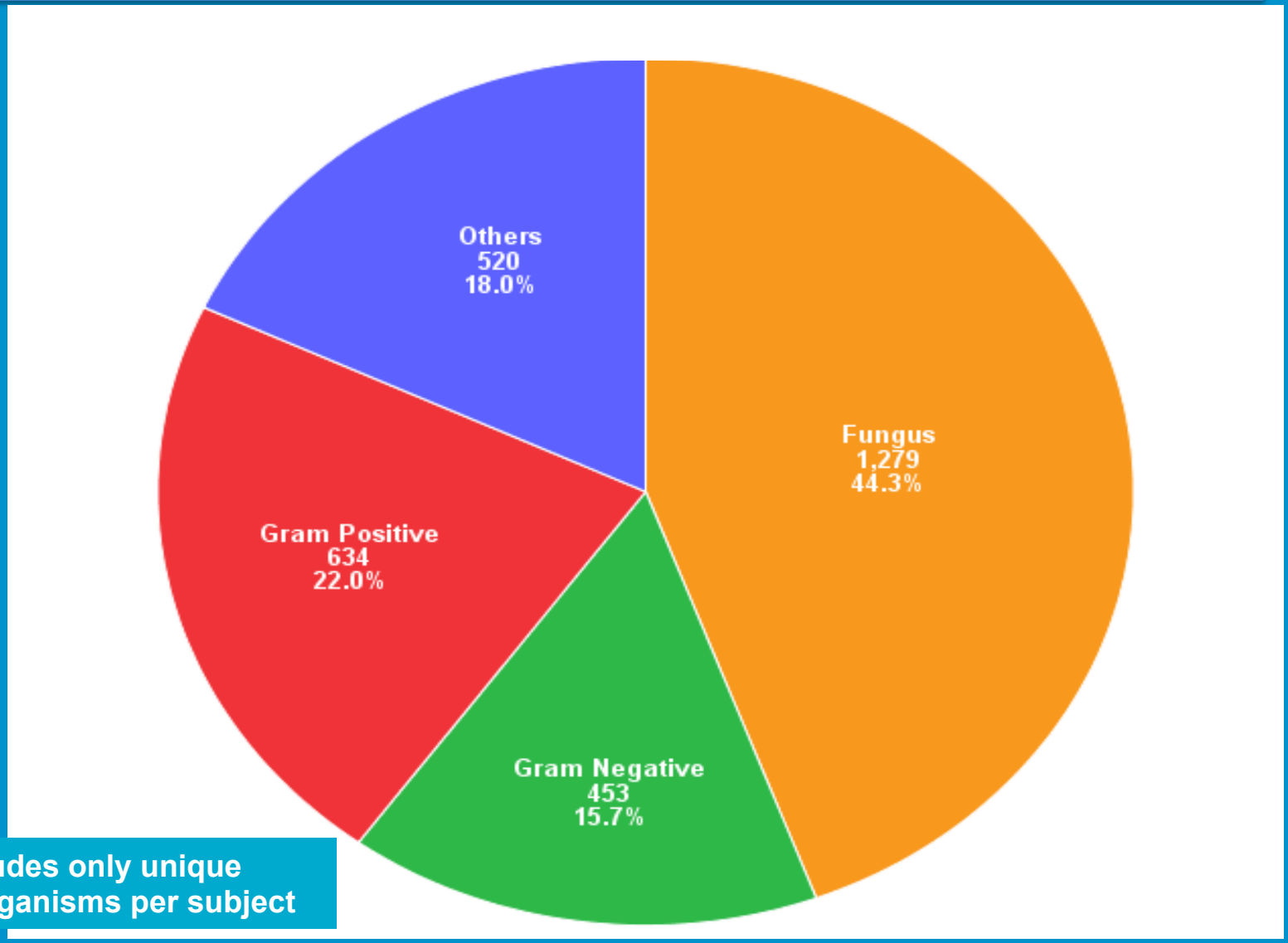
Overall Micro-organism Distribution (N=2,886)



*Includes only unique microorganisms per subject

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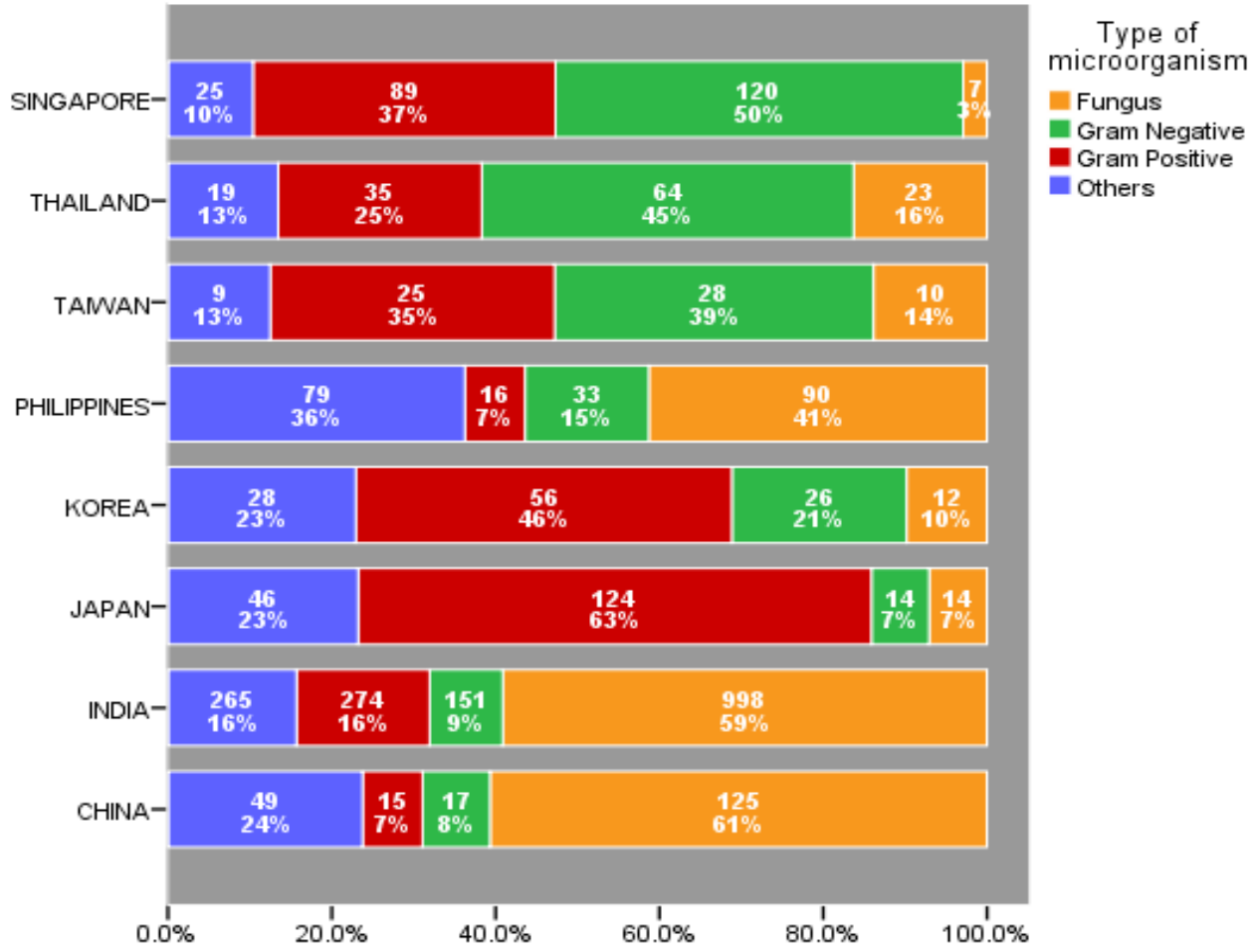
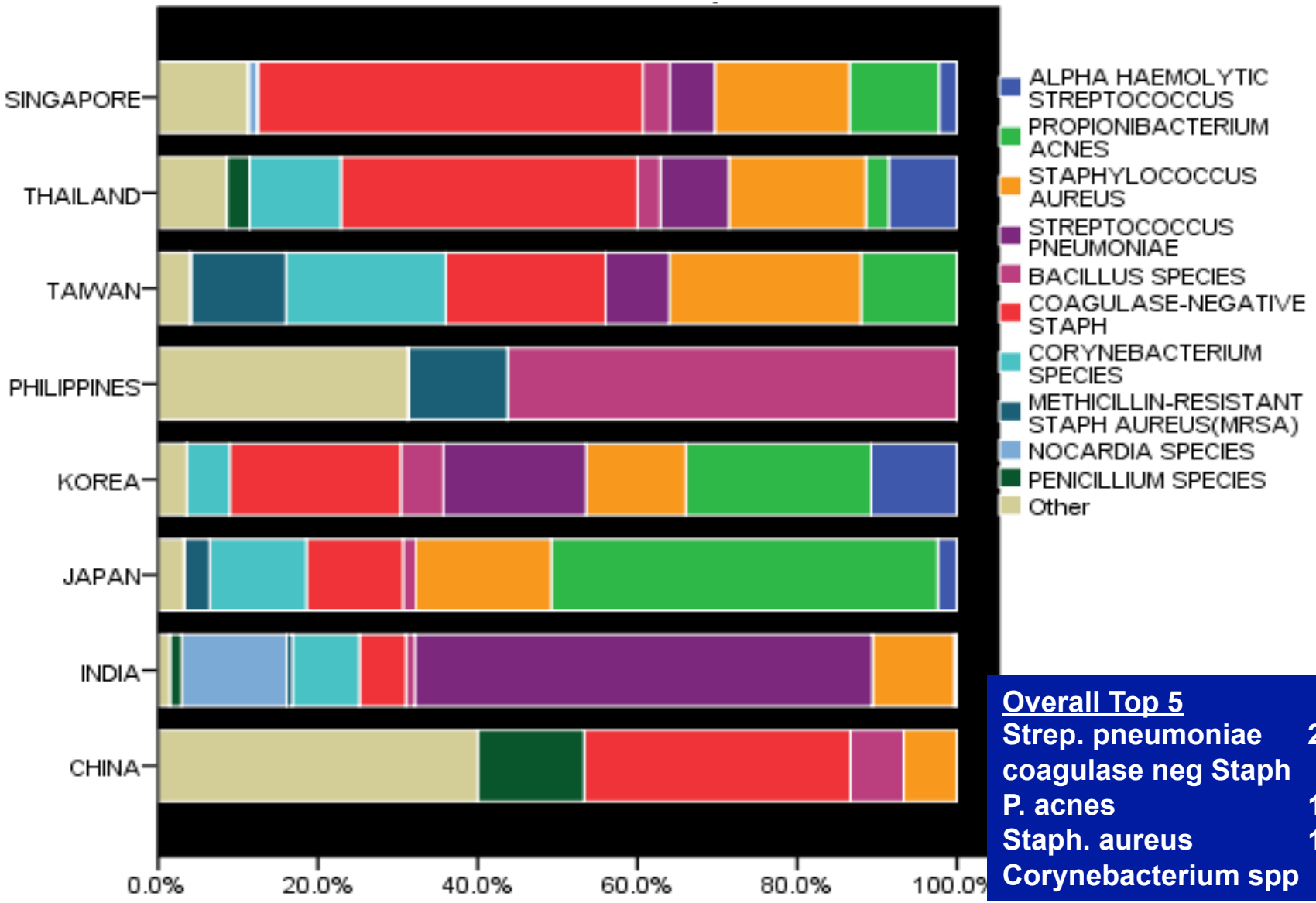
Micro-Organism Distribution by Country



*Includes only unique microorganisms per subject

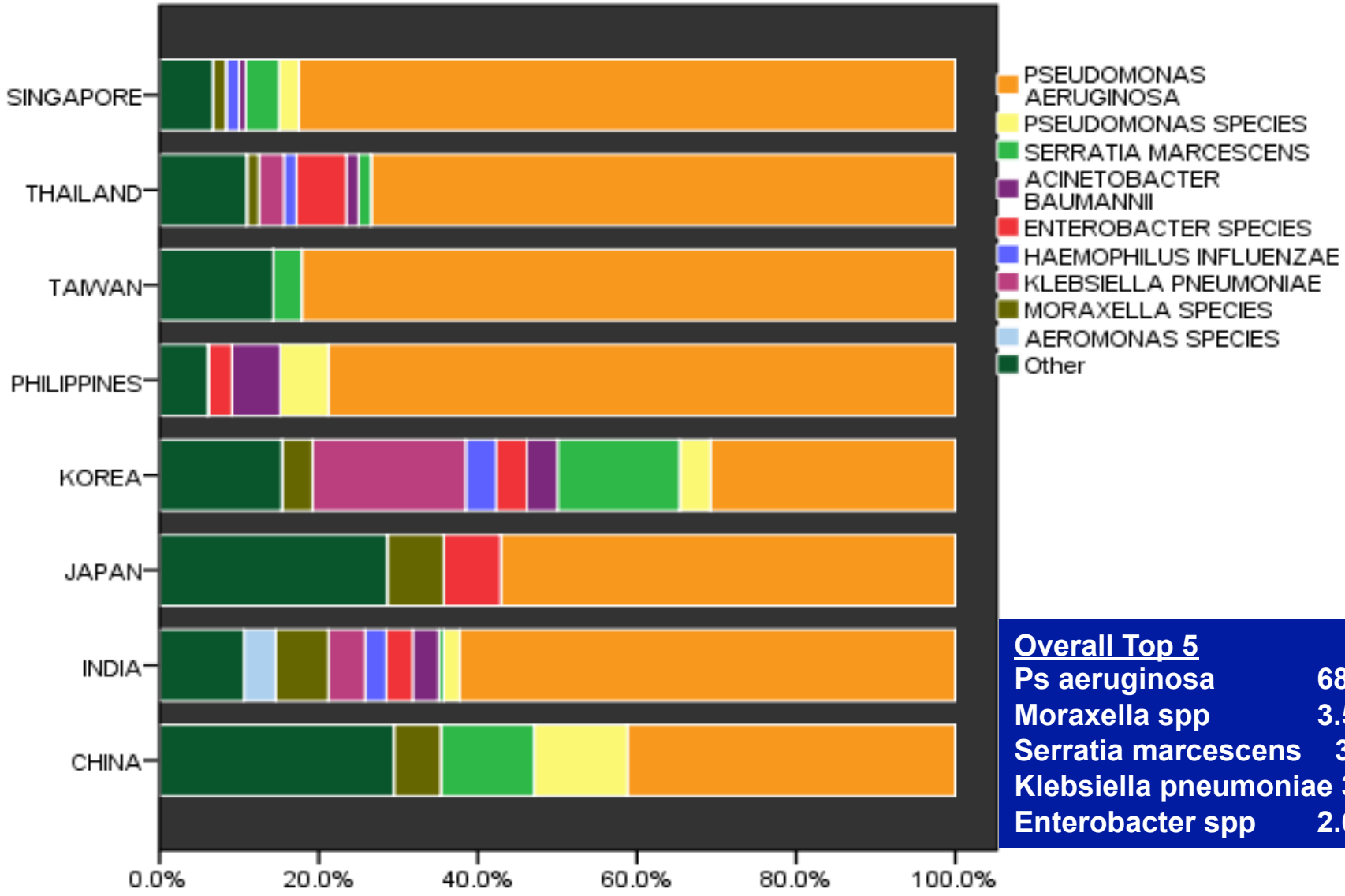
Gram-Positive Bacteria



Overall Top 5

Strep. pneumoniae	27.9% (177)
coagulase neg Staph	17.2% (109)
P. acnes	13.9% (88)
Staph. aureus	13.5% (84)
Corynebacterium spp	7.9% (50)

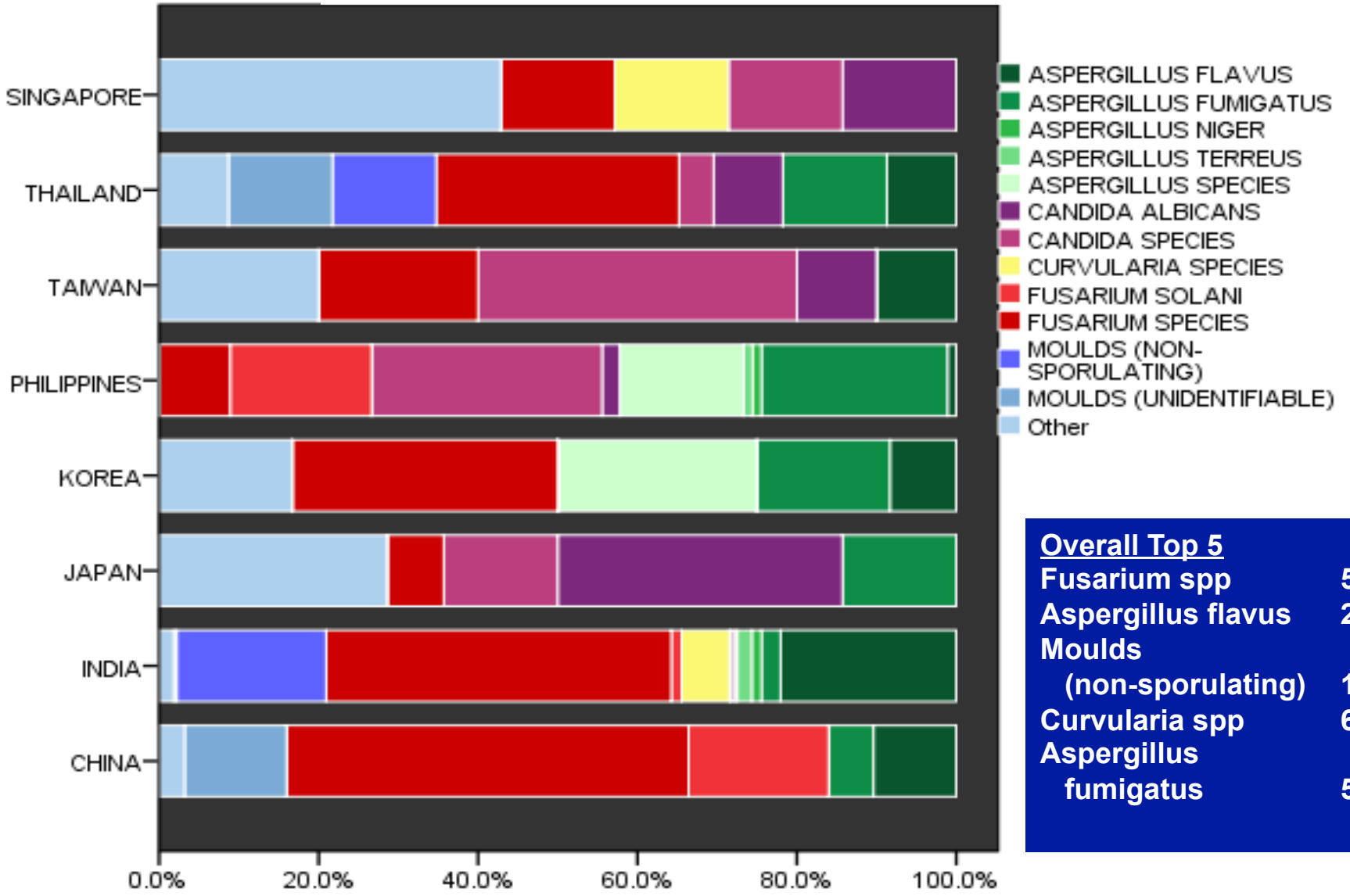
Gram-Negative Bacteria



Overall Top 5

Ps aeruginosa	68.9% (312)
Moraxella spp	3.5% (16)
Serratia marcescens	3.1% (14)
Klebsiella pneumoniae	3.1% (14)
Enterobacter spp	2.6% (12)

Fungus



Overall Top 5

Fusarium spp	**518 (40.5%)**
Aspergillus flavus	**238 (18.6%)**
Moulds (non-sporulating)	**190 (14.9%)**
Curvularia spp	**61 (4.8%)**
Aspergillus fumigatus	**59 (46%)**

Antibiotic Resistance

LVPEI
So that all may see

Ophthalmology

What is the important observation of the surveillance studies?

Antibiotic Resistance

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Ophthalmology

TSN-Ocular isolates

- **The proportion of infections caused by MRSA increased from 29.5% in 2000 to 41.6% in 2005**
- **MRSA were resistant to 3 or more class of antibiotics including fluoroquinolones**

Antibiotic Resistance

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Ocular TRUST

Antibiotic	Status	MIC90	Susceptible		Intermediate		Resistant	
			No.	%	No.	%	No.	%
Ciprofloxacin	MSSA	> 8	131	79.9	1	0.6	32	19.5
	MRSA	> 8	5	15.2	0	0.0	28	84.8
Levofloxacin	MSSA	16	133	81.1	0	0.0	31	18.9
	MRSA	> 16	5	15.2	2	6.1	26	78.8
Gatifloxacin	MSSA	4	133	81.1	0	0.0	31	18.9
	MRSA	> 8	5	15.2	1	3.0	27	81.8
Moxifloxacin	MSSA	4	133	81.1	5	3.0	26	15.9
	MRSA	> 8	5	15.2	3	9.1	25	75.8
Azithromycin	MSSA	> 16	89	54.3	0	0.0	75	45.7
	MRSA	> 16	2	6.1	1	3.0	30	90.9
Penicillin	MSSA	> 1	16	9.8	0	0.0	148	90.2
	MRSA	> 1	0	0.0	0	0.0	33	100
Polymyxin B*	MSSA	> 8	0	0.0	†	†	164	100
	MRSA	> 8	0	0.0	†	†	33	100
Tobramycin‡	MSSA	1	152	92.7	2	1.2	10	6.1
	MRSA	> 32	12	36.4	0	0.0	21	63.6
Trimethoprim	MSSA	2	160	97.6	†	†	4	2.4
	MRSA	2	31	93.9	†	†	2	6.1

Antibiotic Resistance

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ARMOR study

- **No increase or a marginal reduction of MRSA compared to 2005 (39% v/s 41.6%)**
- **Fluoroquinolones showed significant increase in inactivity against all Staph**

Antibiotic Resistance

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Ophthalmology

ARMOR study

- **Resistance among *P. aeruginosa* isolates to cephalosporins, fluoroquinolones & carbepenams is increasing**
- **This is a grave concern specially when only 36.0% of *S. aureus* & 21.5% of CNS being susceptible to entire drug classes**

Antibiotic Resistance

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Ophthalmology

Conclusions

- AMR is a serious problem both for systemic & Ophthalmic infections.
- Resistance among Gram negative organisms and multi-drug resistance are serious threats.
- We need strong antibiotic policies for containing this challenge

Thank you!

LVPEI
So that all may see

L V Prasad Eye Institute

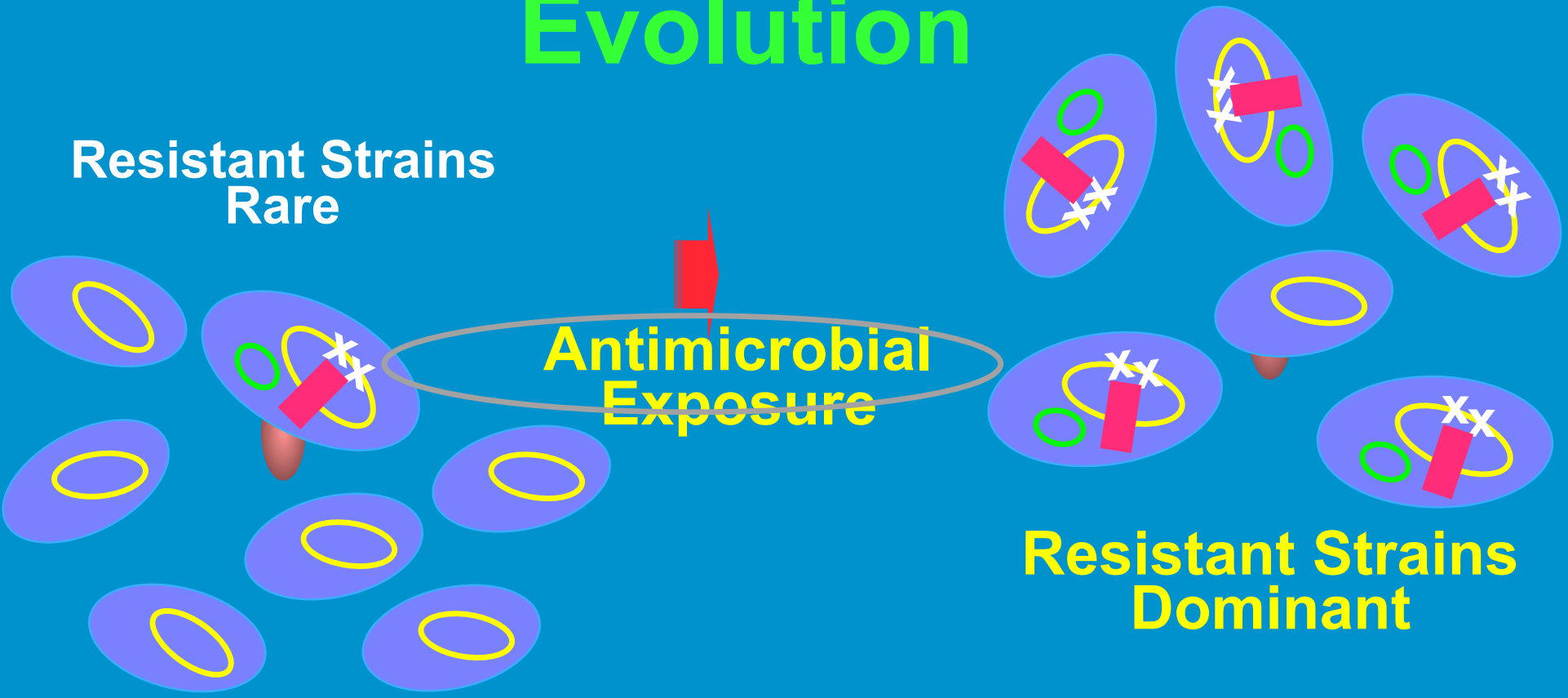
www.lvpei.org

Excellence • Equity • Efficiency

Drugs and Resistance

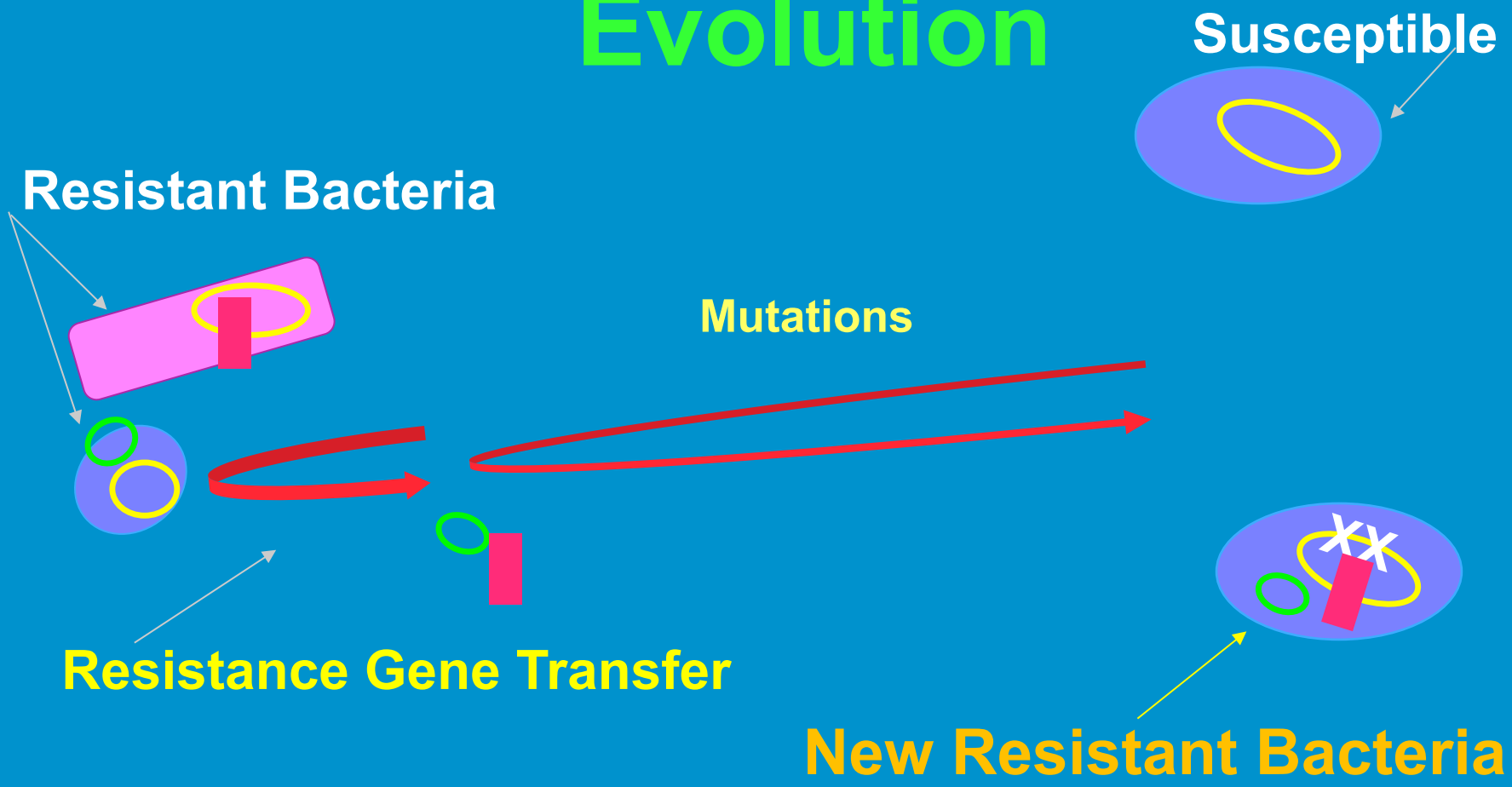
Drug Resistance

Evolution



Drug Resistance

Evolution



Antibiotic Resistance

Ophthalmology

Decreased susceptibility to quinolones in methicillin-resistant *Staphylococcus aureus* isolated from ocular infections at a tertiary eye care centre

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a serious cause of morbidity and mortality worldwide because of its multiple-drug resistance.[1] In the past, MRSA infections were considered as hospital acquired; however, in the 1990s, serious MRSA infections were reported in patients with no previous contact with the healthcare system.[2] Aggressive infections due to MRSA

Year	Methicillin Sensitive (%)	Methicillin Resistant (%)	CI (95%)	P
2006	33 (73.3)	12 (26.7)	13.3-38.7	0.075
2007	59 (64.9)	32 (35.1)	25.2-45.2	
2008	39 (61.9)	24 (38.1)	26.1-50.1	
2009	37 (64.9)	20 (35.0)	25.1–45.2	

Antibiotic Resistance

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"I have been trying to point out that in our lives chance may have an astonishing influence and, if I may offer advice to the young laboratory worker, it would be this—never neglect an extraordinary appearance or happening. It may be—usually is, in fact—a false alarm that leads to nothing, but may on the other hand be the clue provided by fate to lead you to some important advance."

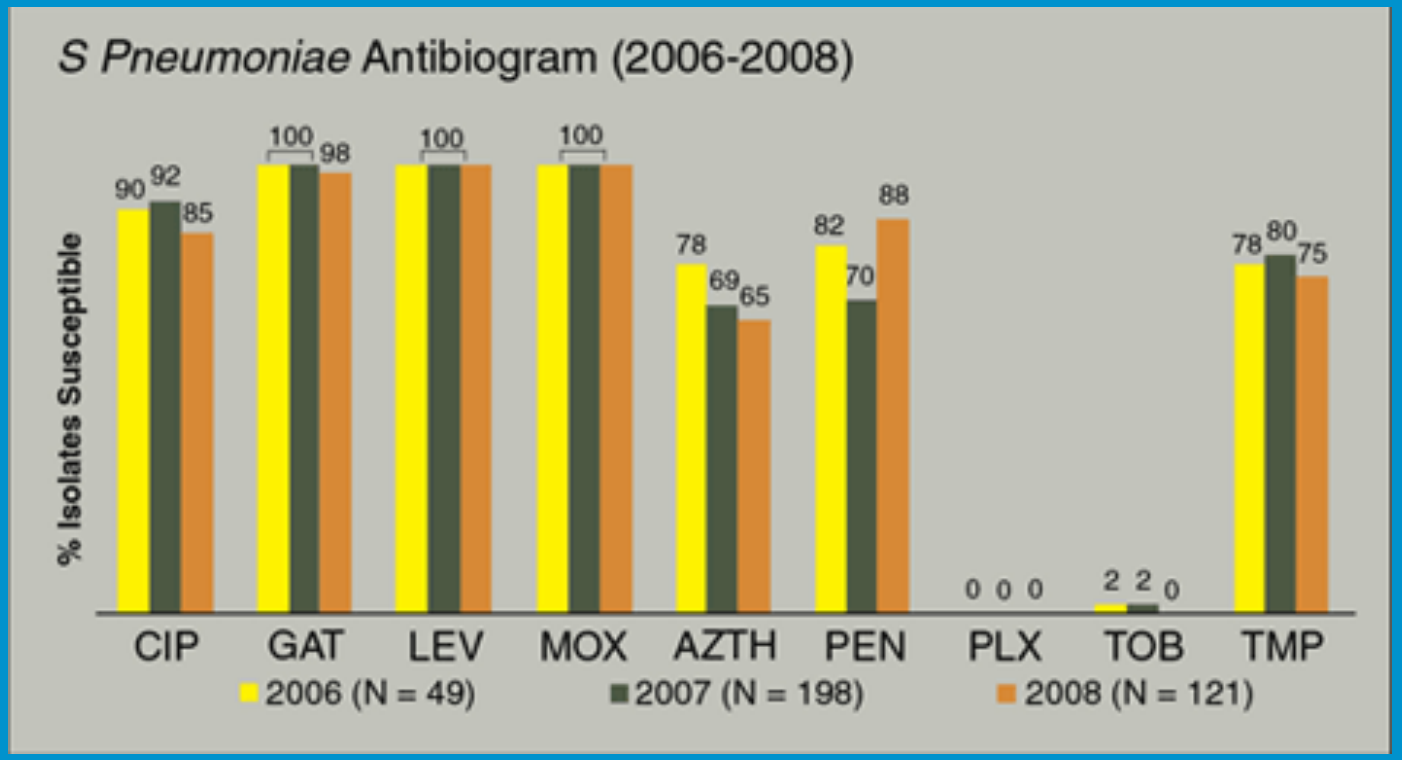
Alexander Fleming

Antibiotic Resistance

LVPEI
So that all may see

Ophthalmology

Ocular TRUST

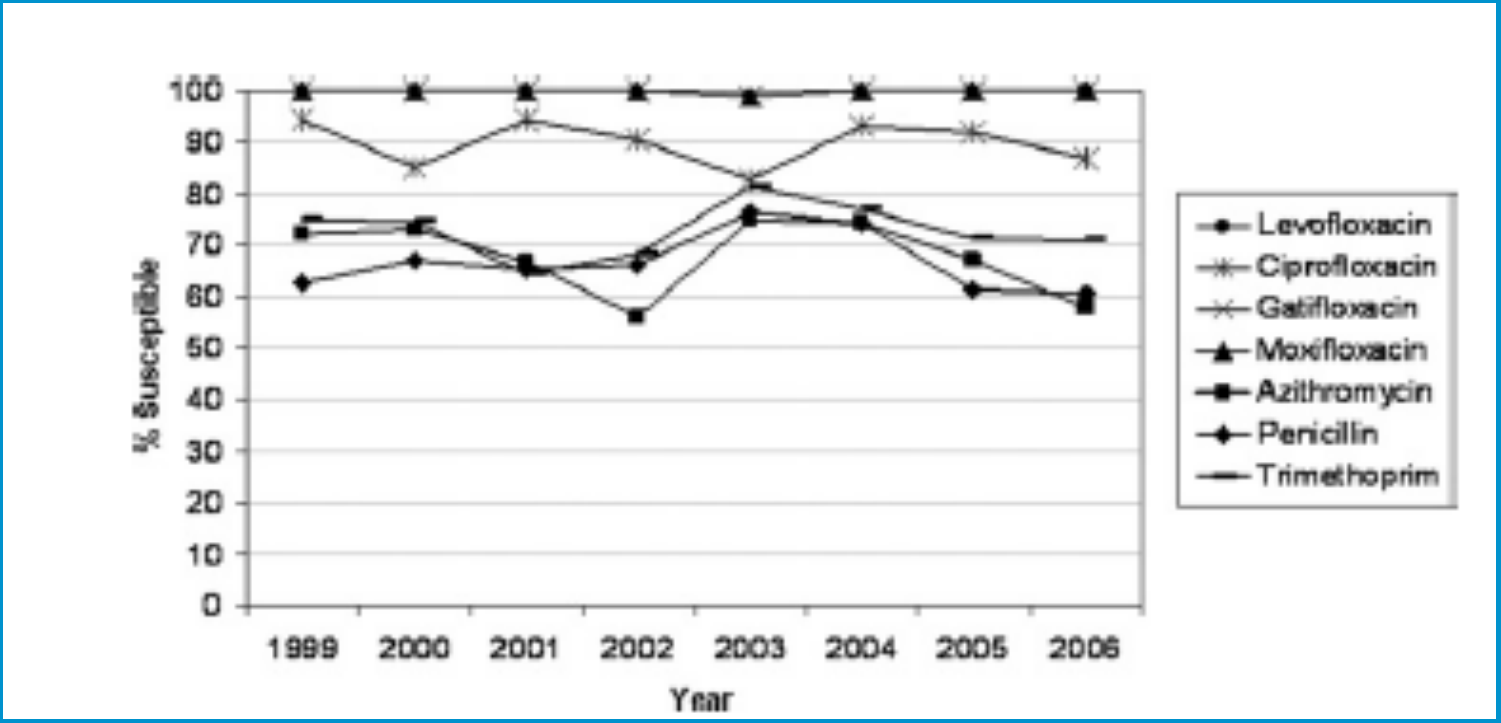


Antibiotic Resistance

LVPEI
So that all may see

Ophthalmology

Ocular TRUST



The University Of Sheffield.

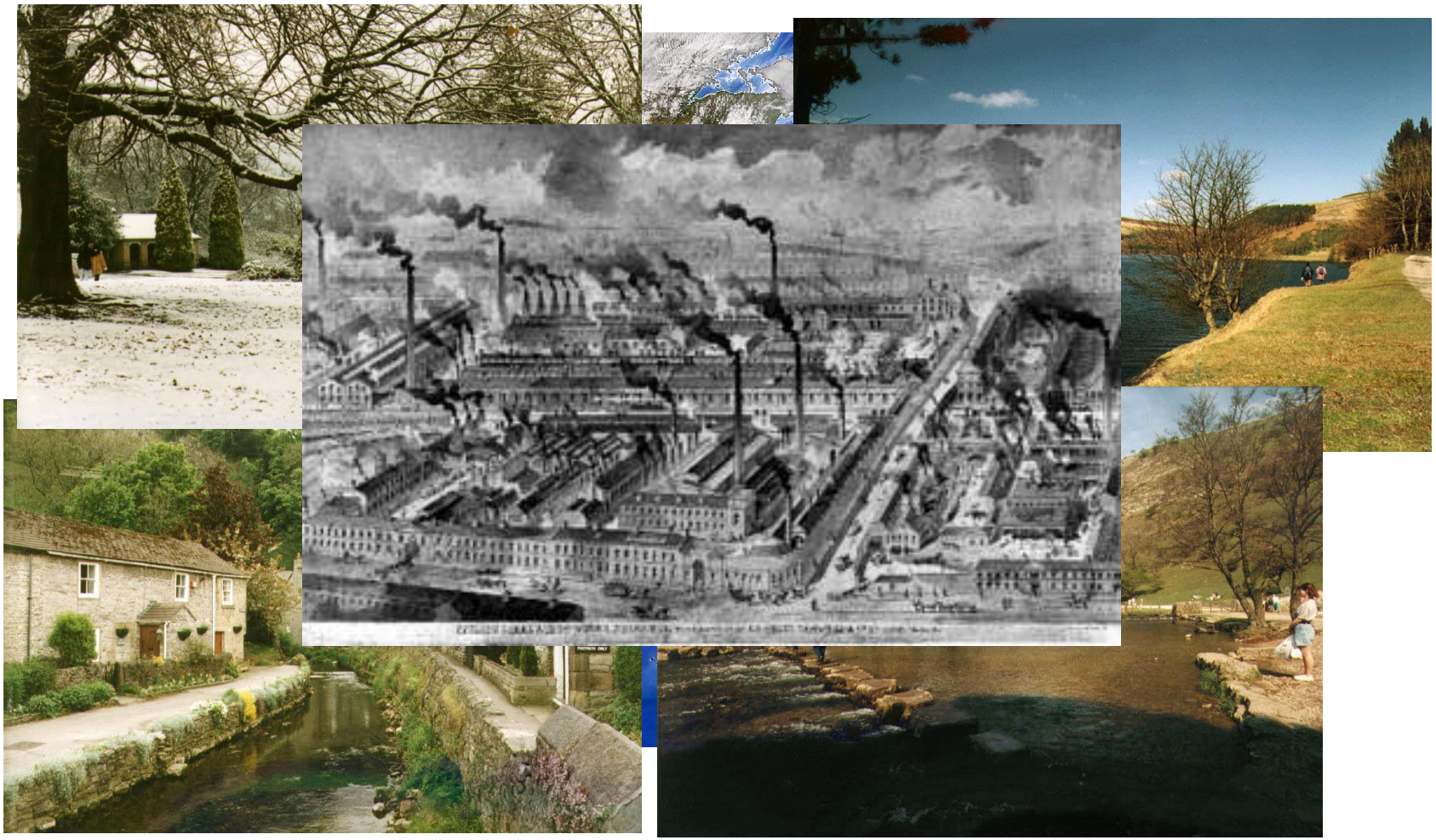
Antibiotic resistance –

Its true biology and global spread

Ian Douglas,

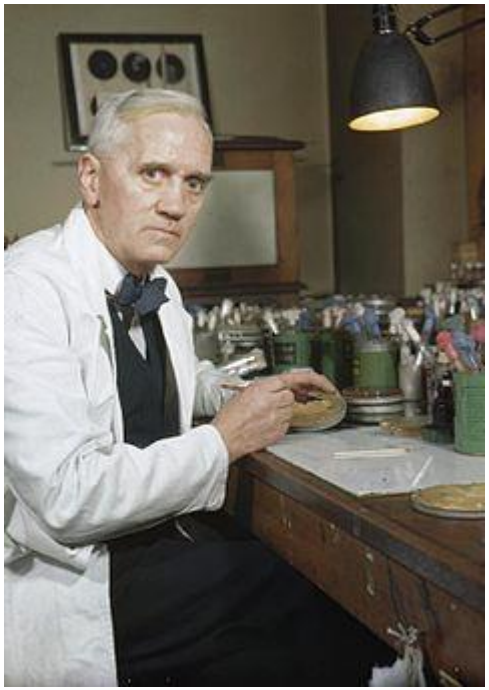
University of Sheffield

Sheffield and district



Antibiotic era

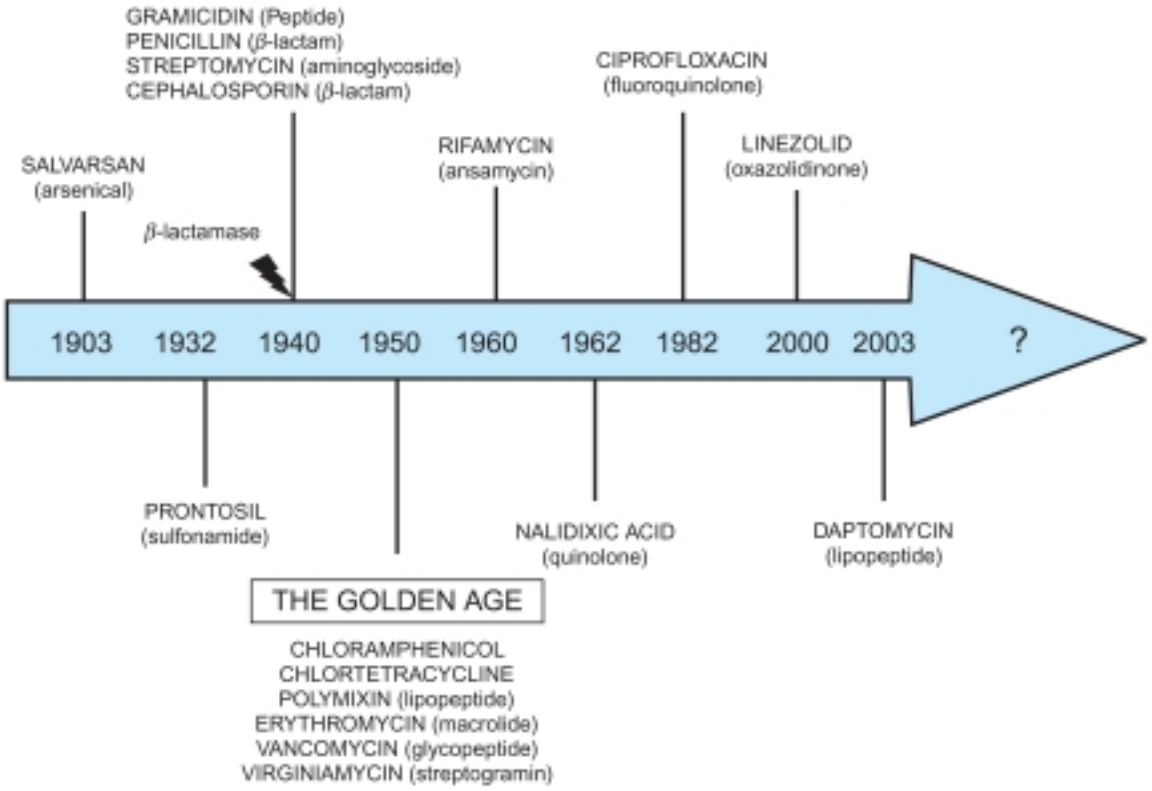
- Survival time of penumonia septicaemia
  - Average no antibiotic – 75% death in 14 days
  - Average with penicillin – 15% death in 14 days



"The time may come when penicillin can be bought by anyone in the shops. Then there is the danger that the ignorant man may easily underdose himself and by exposing his microbes to nonlethal quantities of the drug make them resistant."

Alexander Fleming's Nobel Lecture, 1945

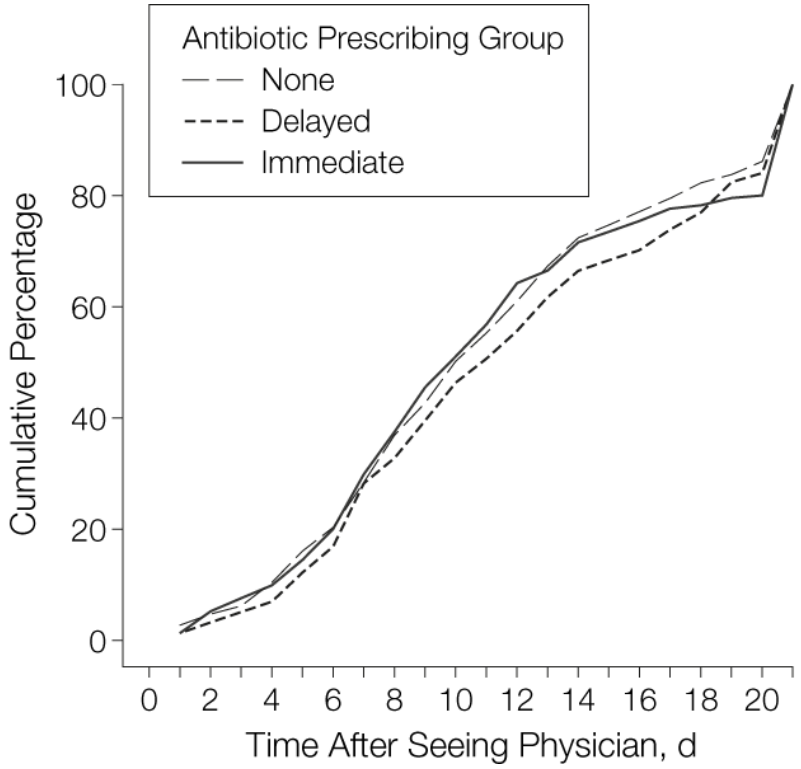
Major classes of antimicrobials and the year of their discovery



J. Davies 2009

Inappropriate use

Resolution of cough



Tanzania – neonatal Gram–ve sepsis

- Survival with inappropriate antibiotic treatment 20% in 14 days
- Survival with appropriate antibiotic treatment 70% in 14 days

Post-antibiotic era

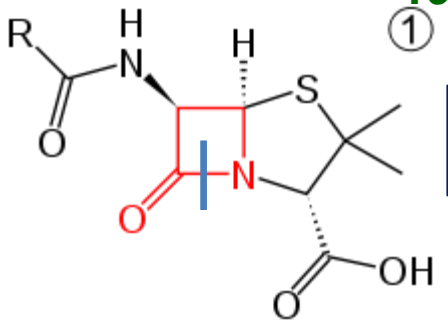
- Inappropriate use selects resistance
  - Exposes resident organisms unnecessarily
- Development of new antimicrobials has been behind the rapid evolution of resistance genes
- In EU 25,000 die from multidrug resistant bacteria per year

Evolution of antimicrobial resistance

penicillin

S.aureus
(90% sens)

(1947)
1950's
①



Penicillin-resistant *S.aureus*
(75% sens)

methicillin

1980's

vancomycin

Methicillin-resistant *S.aureus* (MRSA)
(mecA gene)

We will not stop evolution!

1990's
(5% resist)

Vancomycin resistant *Enterococcus* (*vanA* gene cluster)

1997

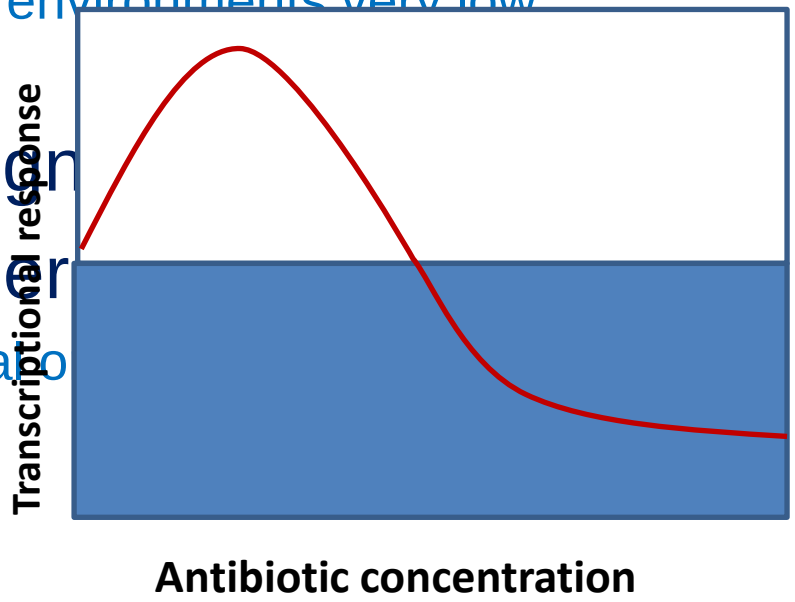
Vancomycin Intermediate resistant MRSA
(not vanA-dependent)

2002

Vancomycin resistant VRSA
(vanA-dependent)

Resistance not entirely Darwinian

- Most antibiotics come from soil organisms (where most pathogens don't live)
  - They have both productive and self-protecting mechanisms
    - Assumed to be a weapon & shield
    - But is their aim to inhibit growth of competitors?
  - Antibiotic concentration in natural environments very low
    - Hormetic effect?
- Antibiotics may serve as sign
- Mutation rates can be higher
  - Can be triggered by environmental
  - Subpopulations
    - 'Bet hedging'

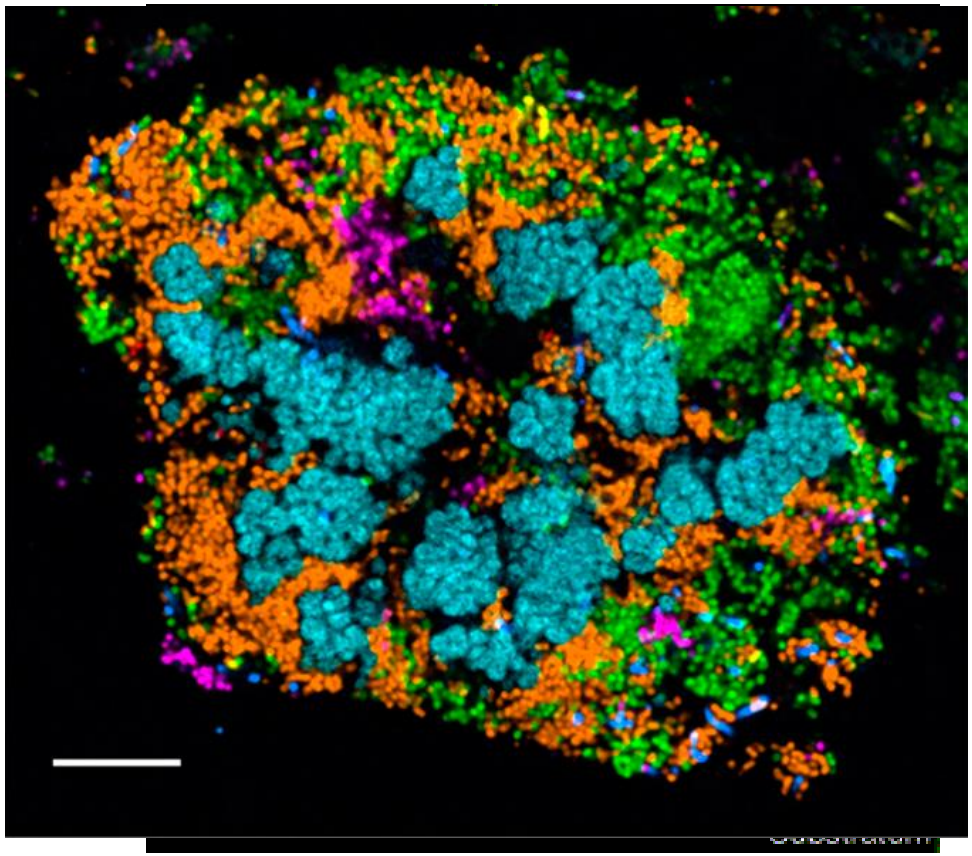


Antibiotic non-responsiveness

- Antibiotic resistance – not killed and continue to grow
  - Develops over time e.g. mutations
  - Clinical problem is due to selective pressure - Darwinian evolution
- Antibiotic tolerance – not killed and not growing
  - There is a spectrum of sensitivities in a population
    - Persisters/dormant cells
  - Re-grow after antibiotics
    - Relevant to biofilms - 3D layered architecture, matrix can bind drug

Biofilms pose particular difficulties

- Green areas - respiratory activity
- Red-orange areas – no respiratory activity
- Red cells are not dead – they are dormant
- Yellow areas - mixture of activities
- Mixed species biofilms offer opportunity for gene transfer

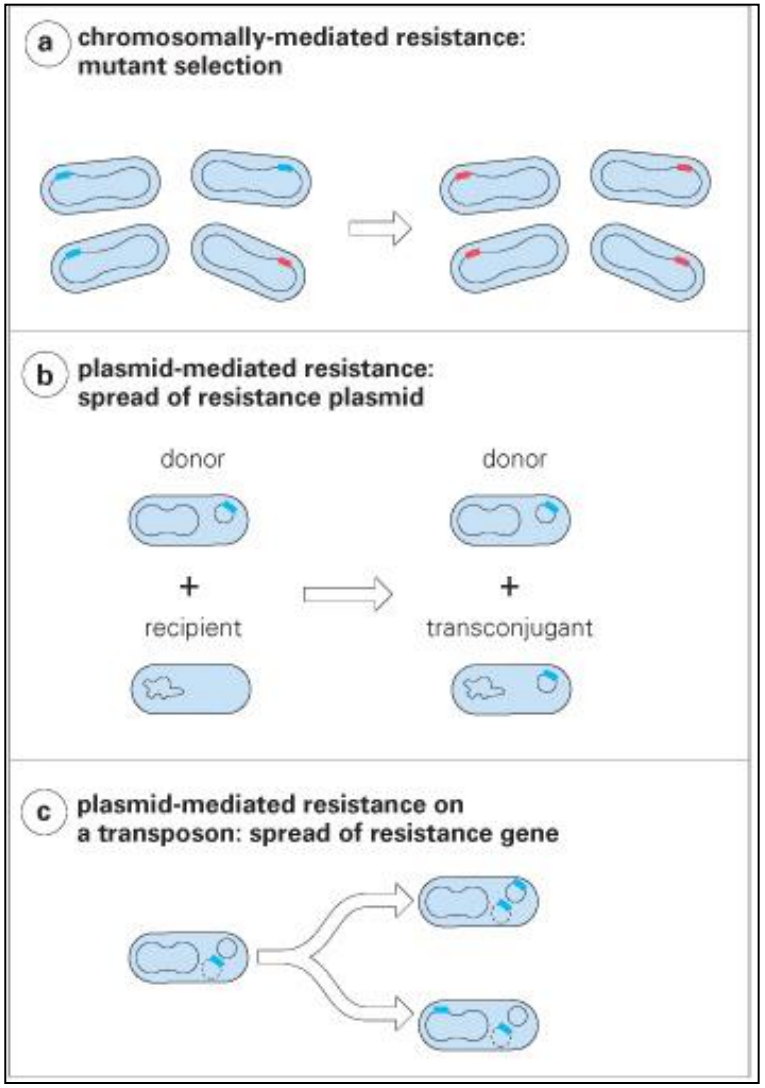


Transfer of resistance

- DNA transfer
  - Plasmid
  - Chromosomal

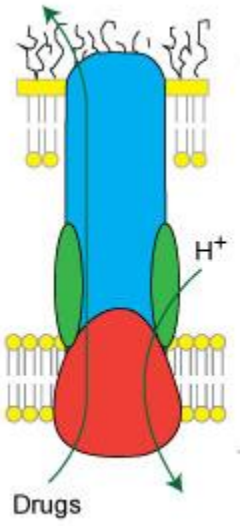
• Pathogens have acquired resistance genes from antibiotic-producing organisms

• Groups of resistance genes assembled into integrons



Antimicrobial resistance

- The target is structurally altered
  - Pencillin-binding proteins
- The target is overproduced
  - Dihydropteroate synthetase and sulphonamide
- The drug is removed
  - Enzymic destruction
    - β-lactamase
    - 3 enzymes modify aminoglycosides
  - Efflux pumps
    - Tetracyclines, quinolones
- The drug cannot penetrate cell



Unexpected mechanisms

- Fluoroquinalone resistance
  - Aminoglycoside-modifying enzyme also acts on a fluoroquinolone - chemically different molecule
  - An N-acetyltransferase that modifies bacterial peptidoglycan can inactivate gentamicin - structural mimicry
- SOS response
  - Defective cell wall synthesis can initiate SOS response,
    - β-lactam antibiotics are extracellular stimuli of the SOS response
  - Transiently halts bacterial cell division,
    - survival of lethal antibiotic exposure.
- Antibiotics can induce competence
  - Chance of acquiring DNA higher when cell death occurring

Broad spectrum antibiotics

- Advantage for clinician
  - Need not worry about aetiological agent
- Longer term disadvantage
  - Selective pressure, not only on aetiological agent but also upon a large fraction of commensals
- Transfer of resistance from commensals to pathogens in man
- Use of antibiotics in farming
  - Transfer from animal commensals to human pathogens

So what are the big issues?

- Lack of regulation and surveillance
  - Athens, 2008
    - 100% of pharmacies sold co-amoxiclav over the counter
    - 53% sold ciprofloxacin despite restrictions
  - Mexico City
    - 30% of antibiotics sold without medical prescription
  - Simple ban?
    - Developing countries - self-prescription often only means of medical treatment
- Lack of or slow development of new agents
  - No financial incentives for pharma
- Lack of sophisticated ways to control infection
  - Knowing the target pathogens quickly
  - Knowing how to control ‘persisters’

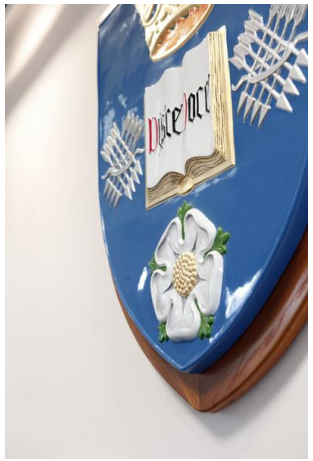
So what are the solutions?

- Modification to clinical practices
  - Reduce the driver for Darwinian evolution
    - Prescribe as infrequently as possible
    - Prescribe the correct dose for the shortest duration to achieve clinical cure
    - Aim the antibiotics at the organisms present
      - Requires knowledge of local sensitivity patterns
- New classes of agents
  - Improve financial incentives for pharma
    - Resurrect abandoned agents
  - Government-sponsored research
- Rapid detection of infection and identification of culprit organisms
- Delivery of high concentrations of targeted agents
  - Systems to get agents at high concentration into biofilms
- Systems to trick bacteria out of dormancy

Conclusion

- Antibiotic Resistance- a collective failure of
  - public policy & global governance
  - research prioritisation
  - the current market system

Thank you



Reasons why antibiotics fail

- Poor patient compliance
  - Can we change human nature?
- Agent does not reach the site
  - Inadequate drainage of abscesses
  - Poor blood supply (inc. foreign body)
  - Inadequate duration
- Impaired defences
  - Immunocompromised (& bacteriostatic)
- Inappropriate agent - resistance
  - Inherent; acquired (mutation, plasmids)

Layer-by-layer self-assembled nanoparticles and thin films for topical drug delivery applications

V. Vamsi Krishna Venuganti, PhD

Assistant Professor

Department of Pharmacy

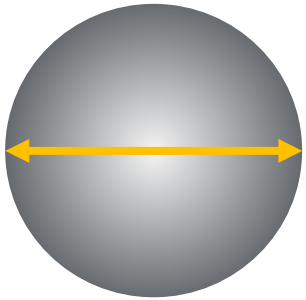
BITS Pilani, Hyderabad Campus

15th March, 2016

BITS Pilani

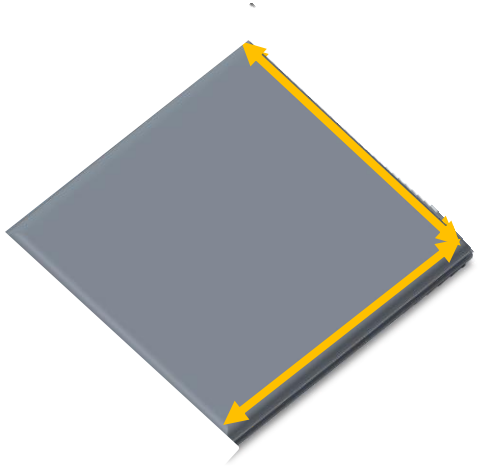
Hyderabad Campus

Drug Delivery Platforms

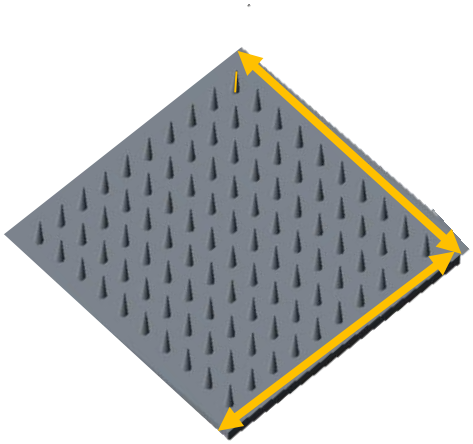


Nanoparticle-based system

- LbL Nanoparticles
- Liposomes

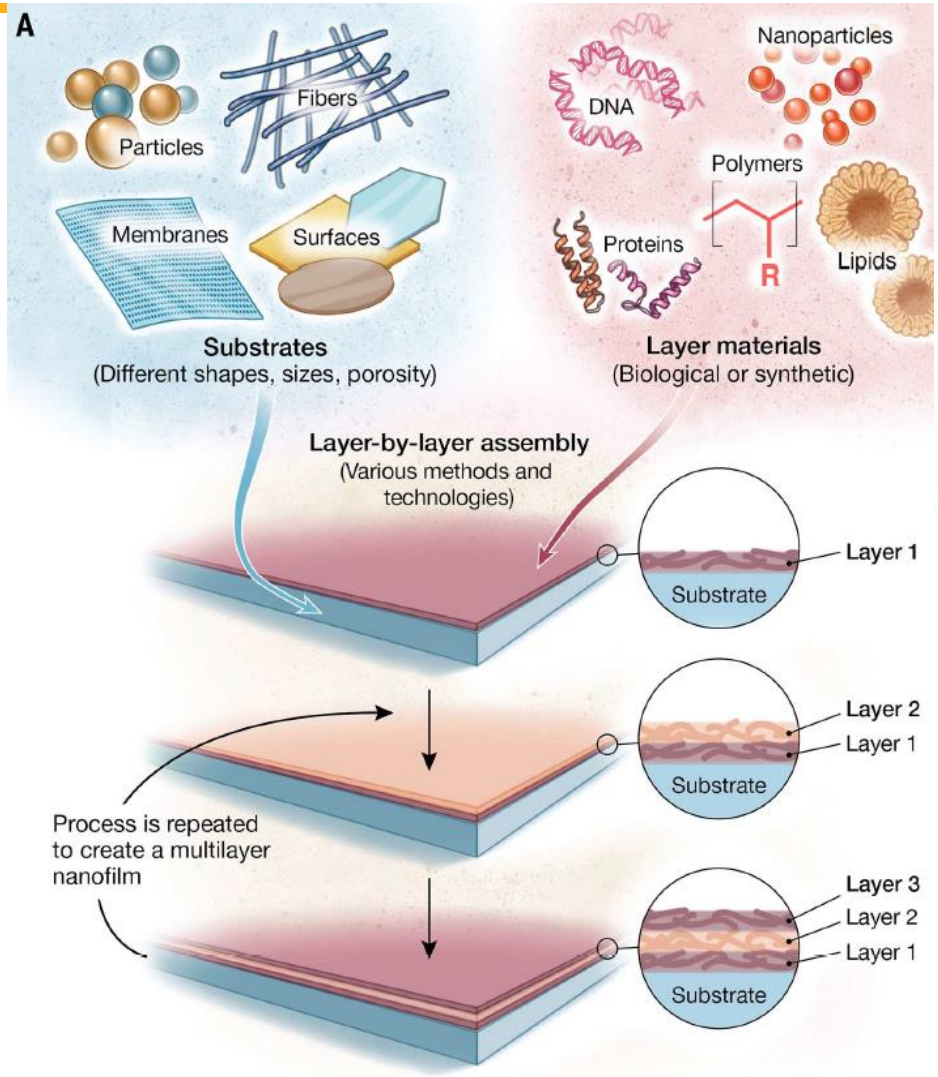


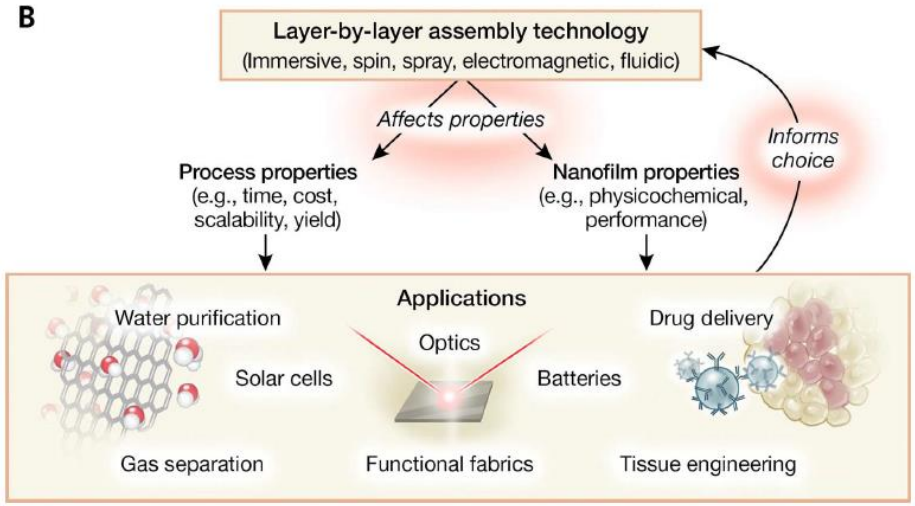
Polymeric thin films



Microneedle array

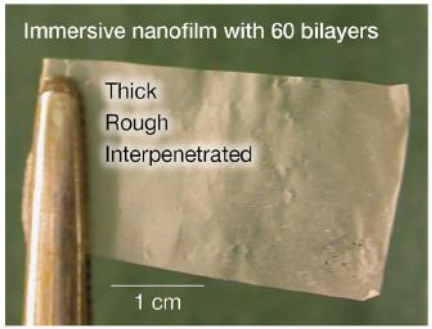
Layer-by-layer assembly

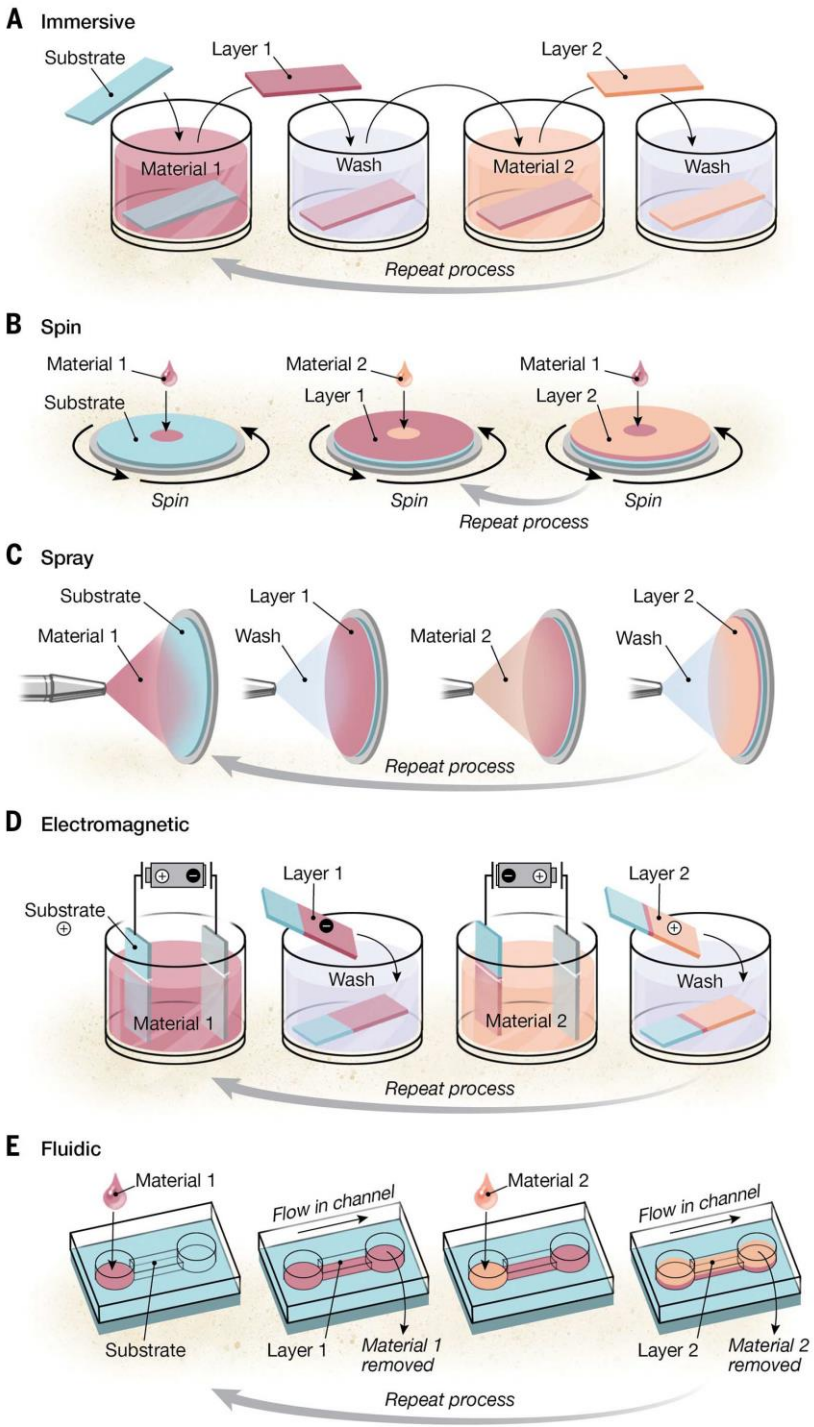




J. J. Richardson et al., Science 348, aaa2491 (2015)

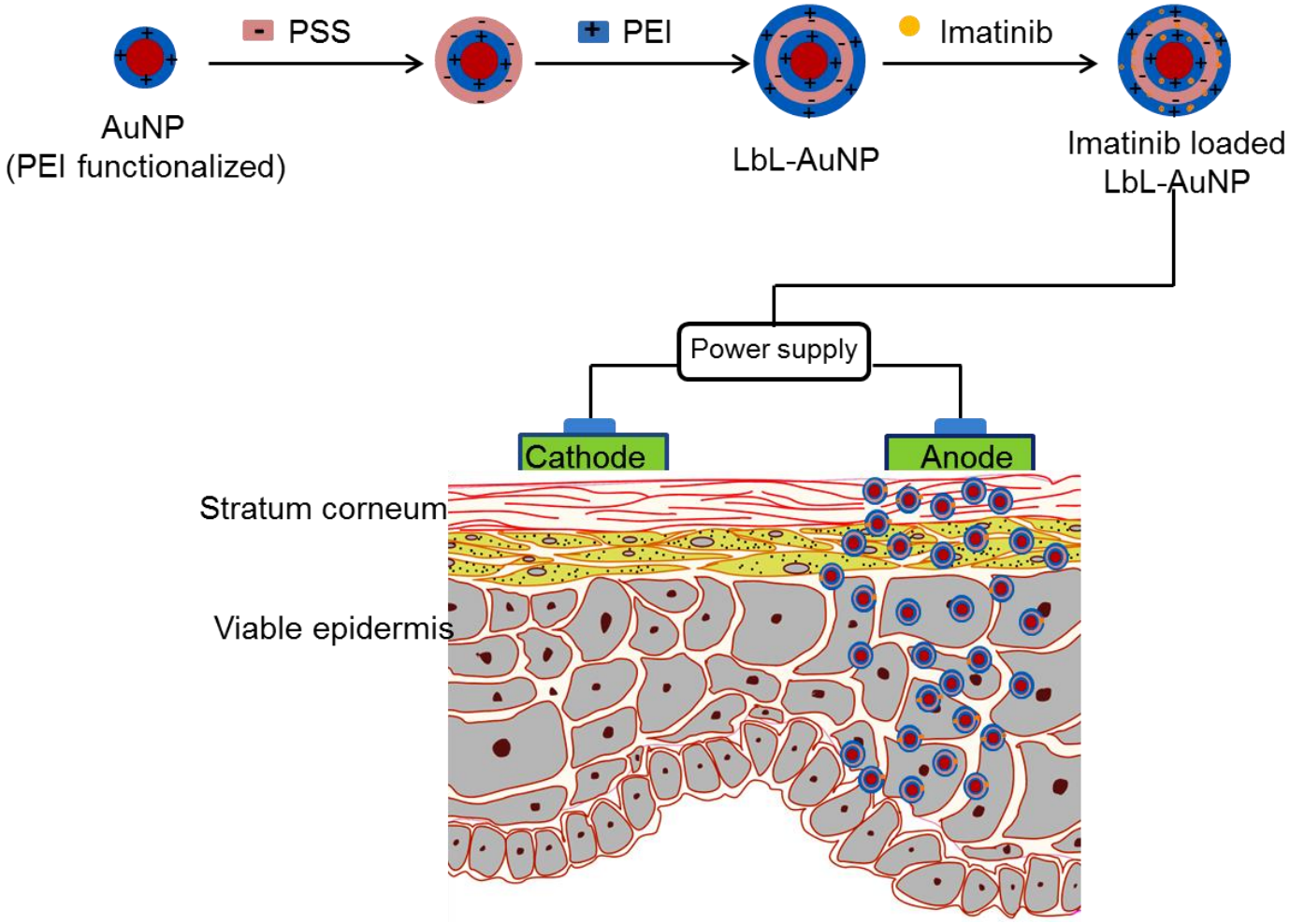
Layer-by-layer assembly technologies



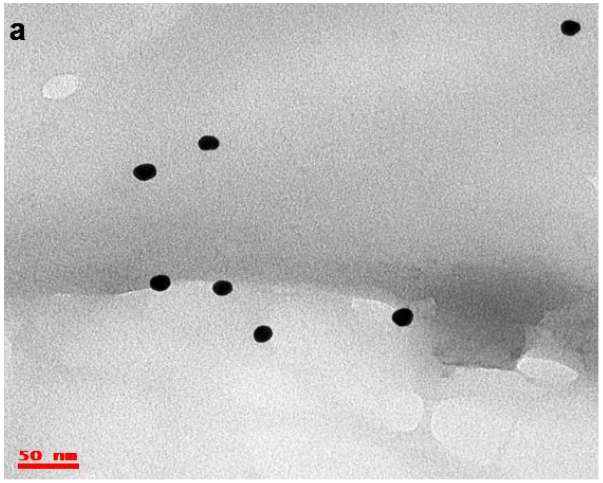


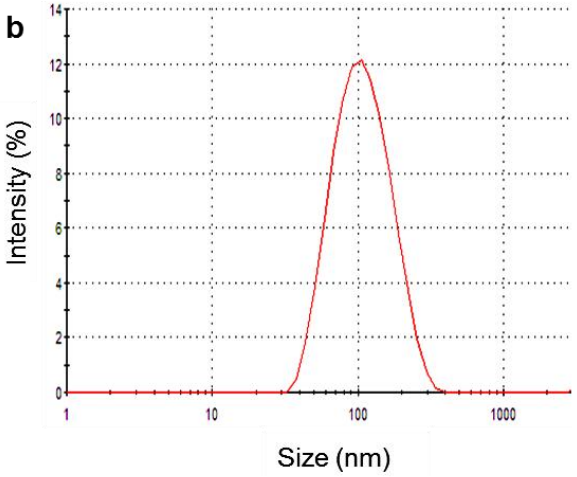
J. J. Richardson et al., Science 348, aaa2491 (2015)

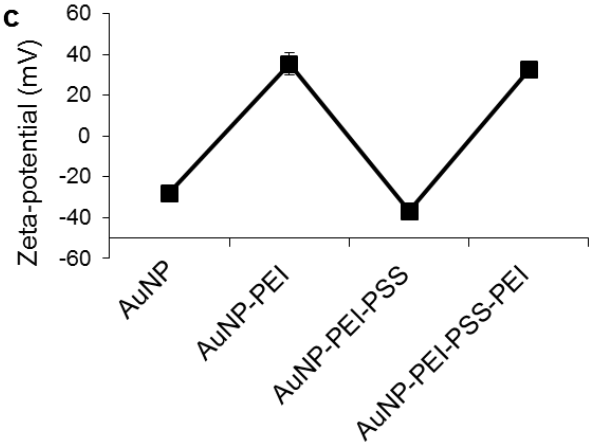
Scheme for LbL Nanoparticle skin application

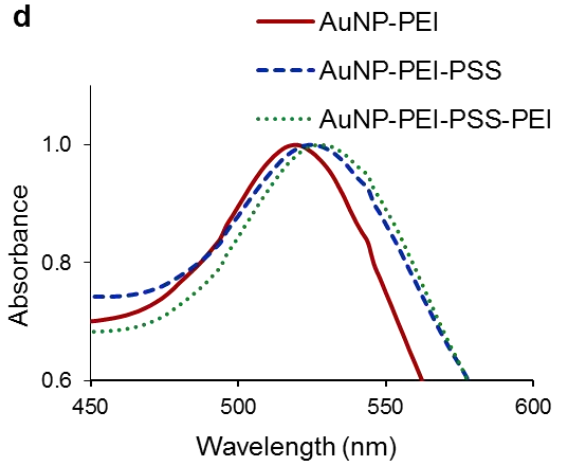


Characterization of LbL-AuNP



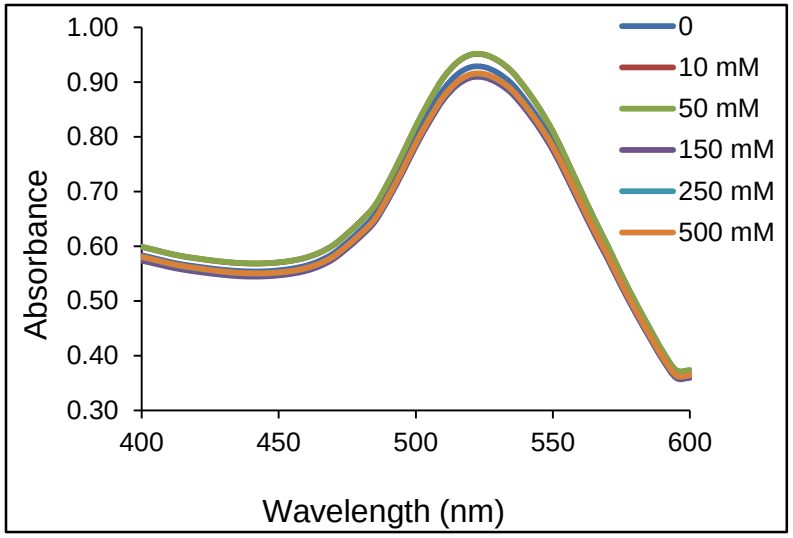


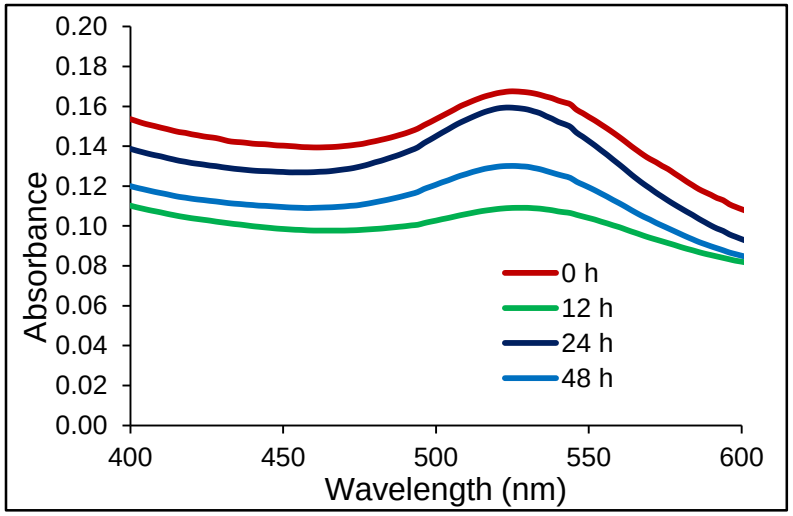


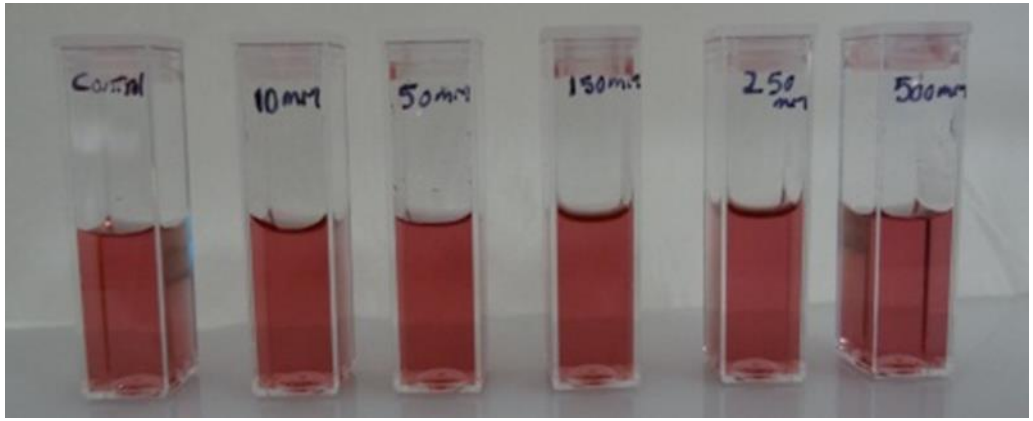


Labala et al. Molecular Pharmaceutics 2015, 2: 878-888

Stability of LbL-AuNP



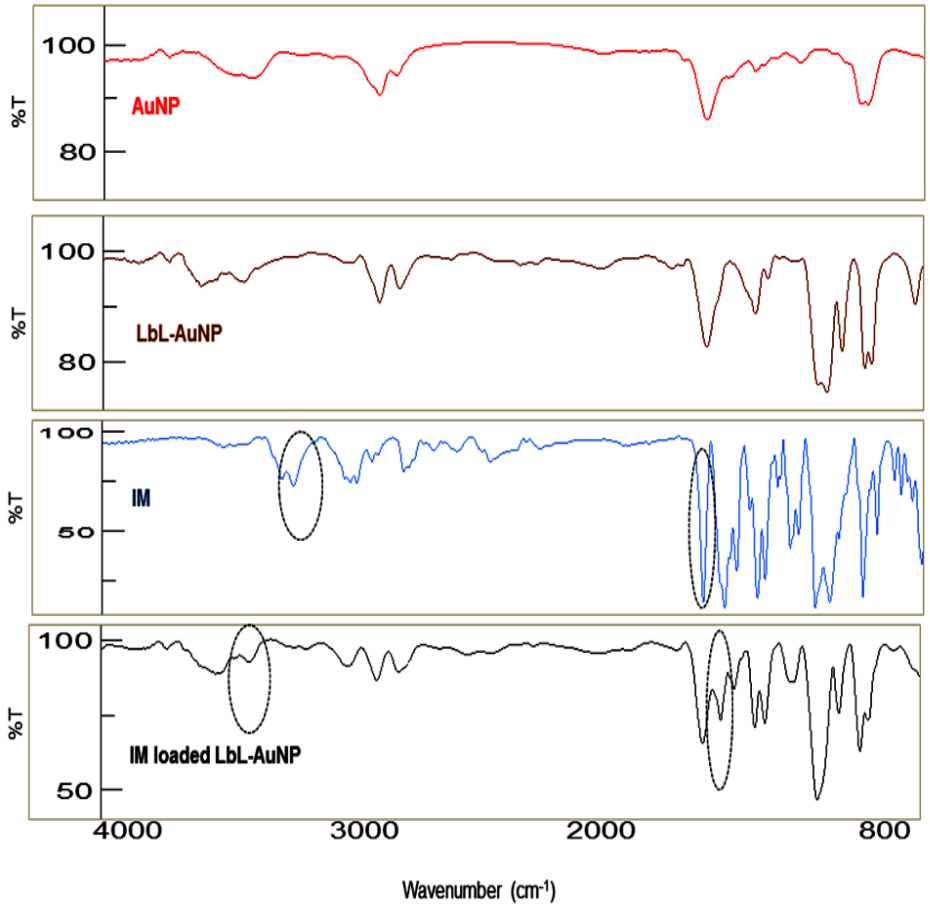


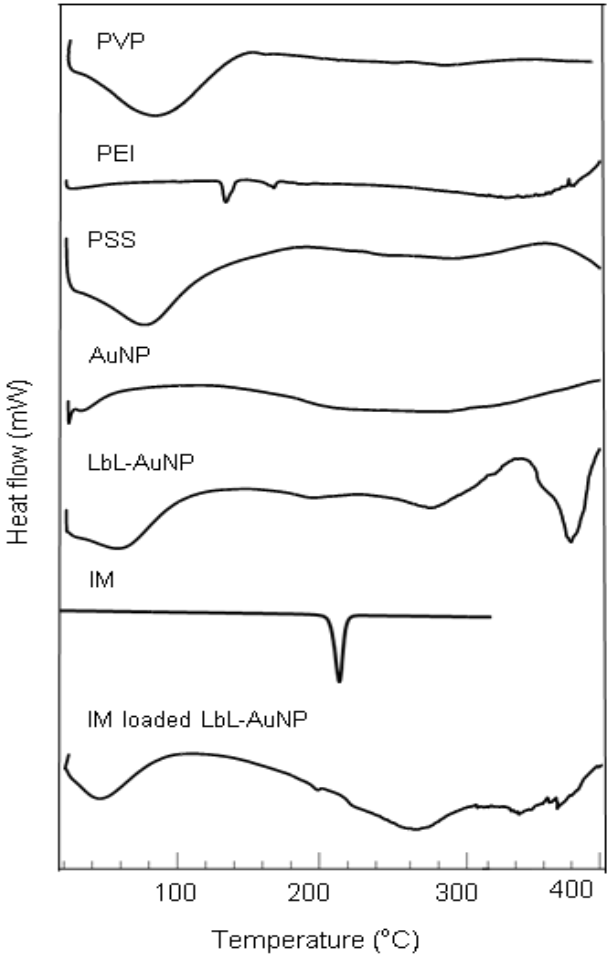


a. Effect of NaCl concentration on localized surface plasmon resonance (SPR) wavelength of AuNP. **b.** Photograph of effect of NaCl concentration on stability of AuNP.

Labala et al. Molecular Pharmaceutics 2015, 2: 878-888

Imatinib loaded LbL-AuNP





Labala et al. Molecular Pharmaceutics 2015, 2: 878-888

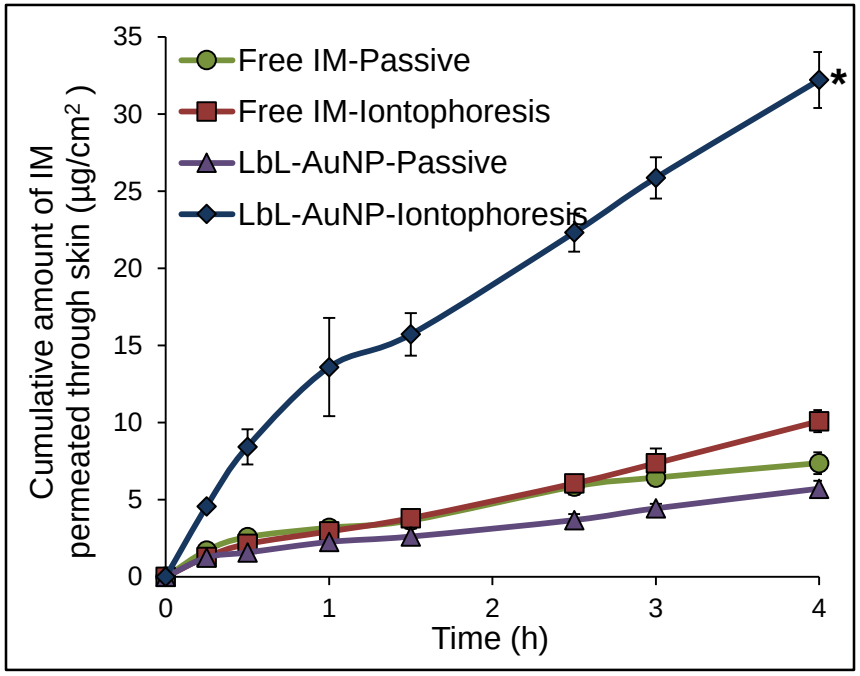
Table 2. Particle Size and Zeta-Potential Measurements of IM Loaded LbL-AuNP Dispersed in Phosphate Buffered Saline (PBS)[a]

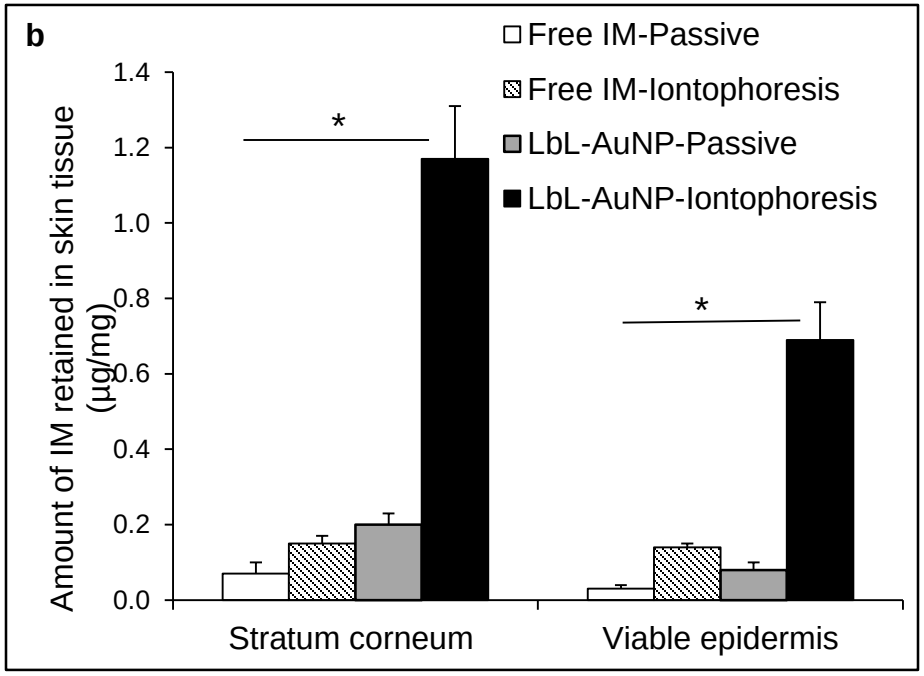
parameter	incubation time (h)	IM loaded LbL-AuNP	IM loaded LbL-AuNP in presence of 0.47 mA electric current
size (nm)	0	94.3 ± 4.4 (0.37 ± 0.03)[b]	94.3 ± 4.4 (0.37 ± 0.03)
	1	115.3 ± 2.2 (0.24 ± 0.02)	114.8 ± 3.2 (0.29 ± 0.05)
	2	120.1 ± 6.6 (0.24 ± 0.01)	117.3 ± 1.0 (0.28 ± 0.03)
	3	116.5 ± 2.1 (0.27 ± 0.01)	123.8 ± 5.1 (0.37 ± 0.02)
	4	121.1 ± 5.0 (0.28 ± 0.02)	135.3 ± 1.9 (0.37 ± 0.02)
zeta-potential (mV)	0	32.0 ± 4.6	32.0 ± 4.6
	1	11.7 ± 1.2	13.2 ± 0.6
	2	12.3 ± 3.1	13.5 ± 2.0
	3	13.4 ± 0.4	12.3 ± 1.7
	4	14.1 ± 1.6	12.3 ± 1.6

[a]Data are presented as mean ± standard deviation (n = 3.) [b]Values in parentheses represent polydispersity index (PDI) ± standard deviation.

Labala et al. Molecular Pharmaceutics 2015, 2: 878-888

Skin permeation of IM loaded LbL-AuNP





Labala et al. Molecular Pharmaceutics 2015, 2: 878-888

Skin permeation of IM loaded LbL-AuNP

innovate achieve lead

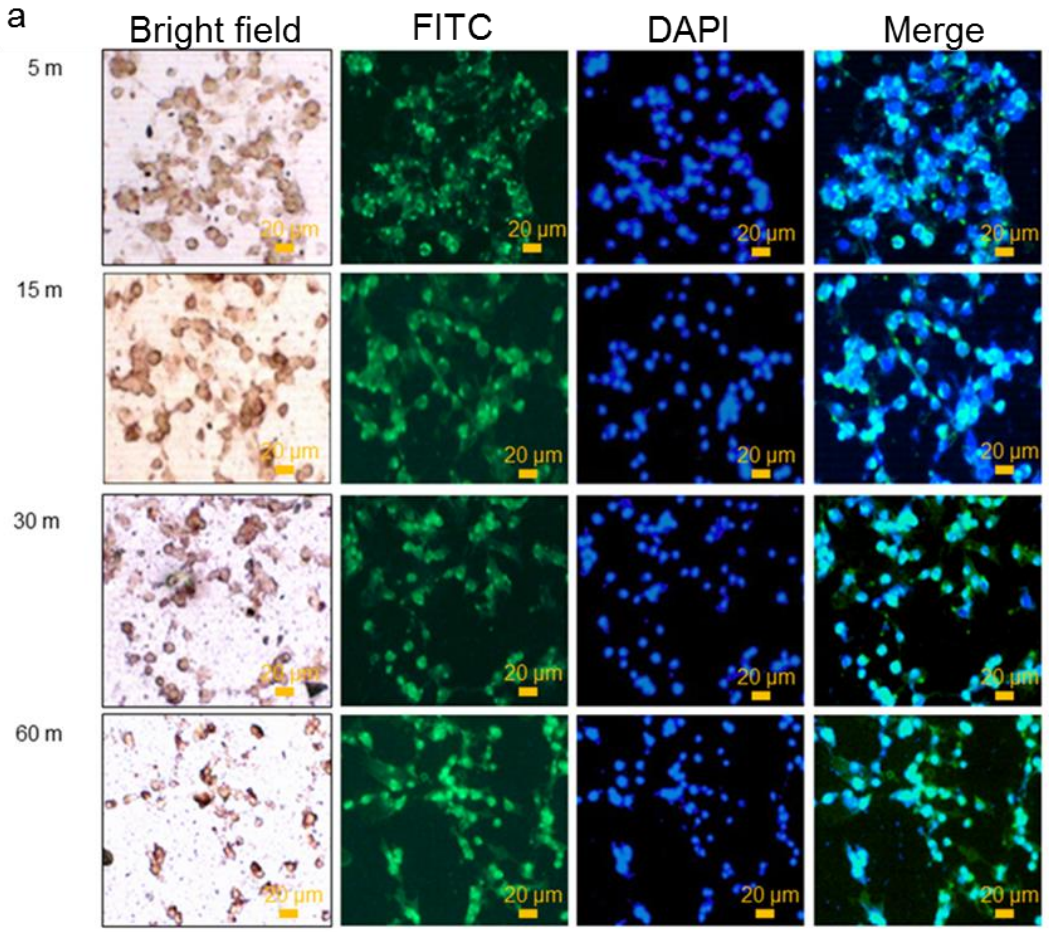
Table 1. Skin Permeation Parameters of IM Permeated through LbL-AuNP with and without Iontophoresis in Comparison with Free Drug[a]

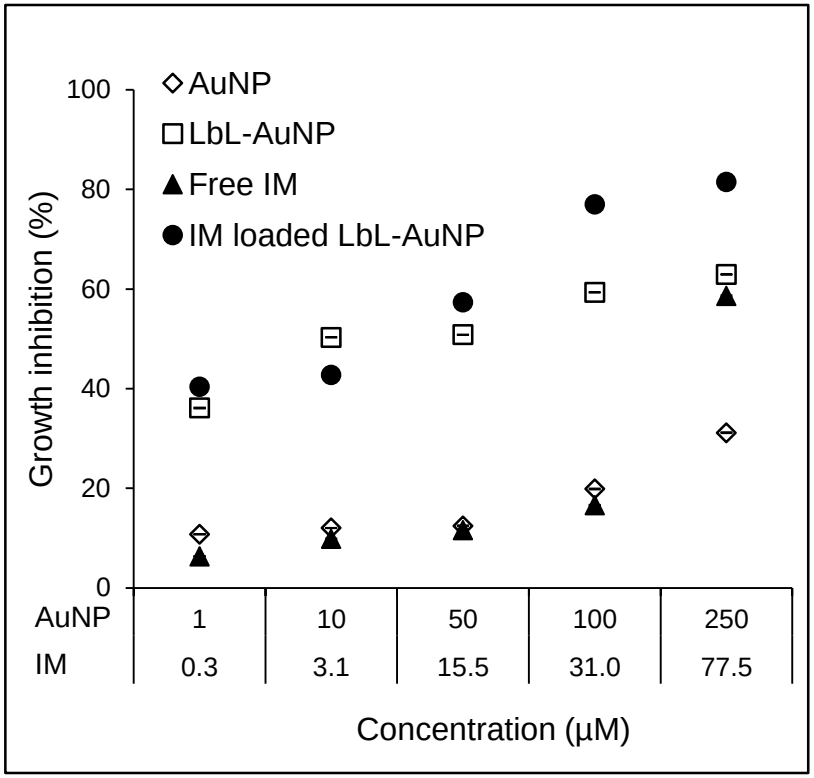
		free IM		IM loaded LbL-AuNP	
	parameter	passive	iontophoresis	passive	iontophoresis
4 h					
	flux ($\mu g/cm^2/h$)	0.94 ± 0.18	1.64 ± 0.21	1.1 ± 0.30	6.37 ± 0.66*
	lag time (h)	3.38 ± 0.45	0.82 ± 0.17	2.20 ± 0.04	0.96 ± 0.51
	Q_4 ($\mu g/cm^2$)	6.90 ± 1.12	10.10 ± 0.71	5.47 ± 0.65	31.68 ± 4.80*
	permeability coeff (cm^2/h)	0.002 ± 0.0004	0.004 ± 0.0005	0.003 ± 0.001	0.017 ± 0.002*
48 h					
	flux ($\mu g/cm^2/h$)	1.03 ± 0.19	NA	1.2 ± 0.28	NA
	lag time (h)	3.22 ± 1.08		3.15 ± 0.35	
	Q_{48} ($\mu g/cm^2$)	267.6 ± 64.6		129.83 ± 6.55	
	permeability coeff (cm^2/h)	0.0026 ± 0.0005		0.003 ± 0.001	

[a] Q_4 and Q_{48} represent cumulative amount of IM permeated across skin after 4 and 48 h, respectively. Data are presented as mean ($n = 4$) ± standard deviation. * represents that the values are significantly ($p < 0.05$) different compared with all other values within the parameter.

Labala et al. Molecular Pharmaceutics 2015, 2: 878-888

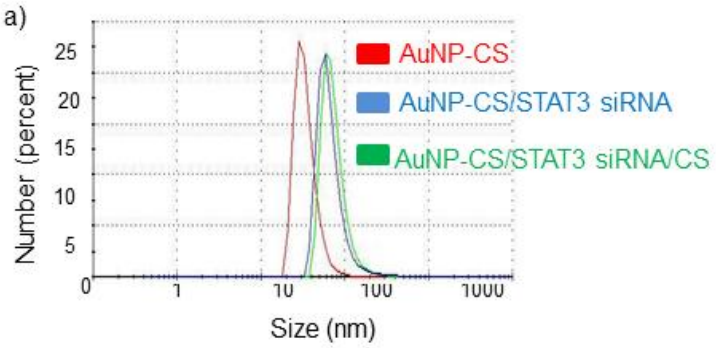
Cell uptake and cell viability studies

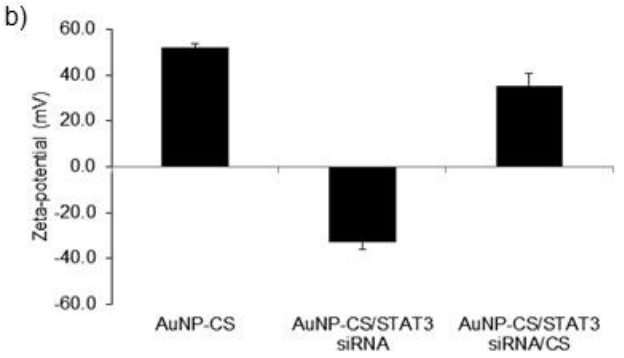


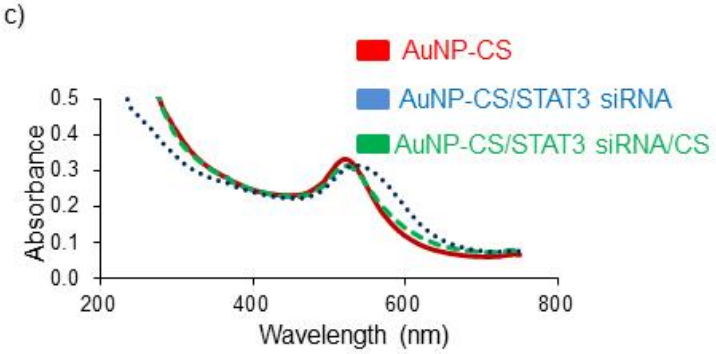


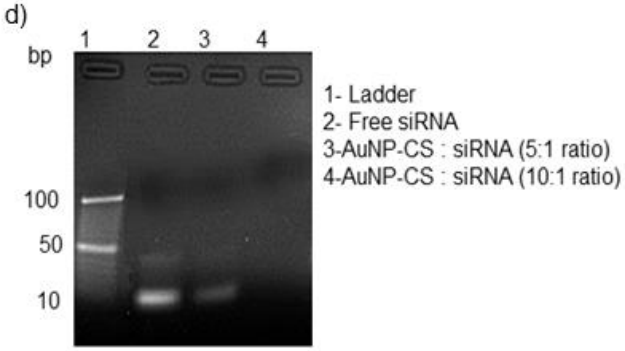
Labala et al. Molecular Pharmaceutics 2015, 2: 878-888

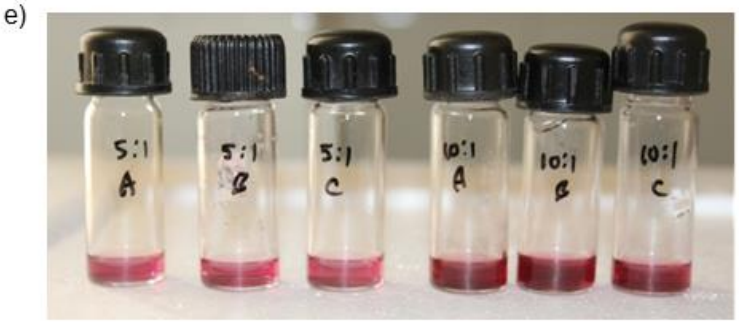
siRNA loaded LbL-AuNP



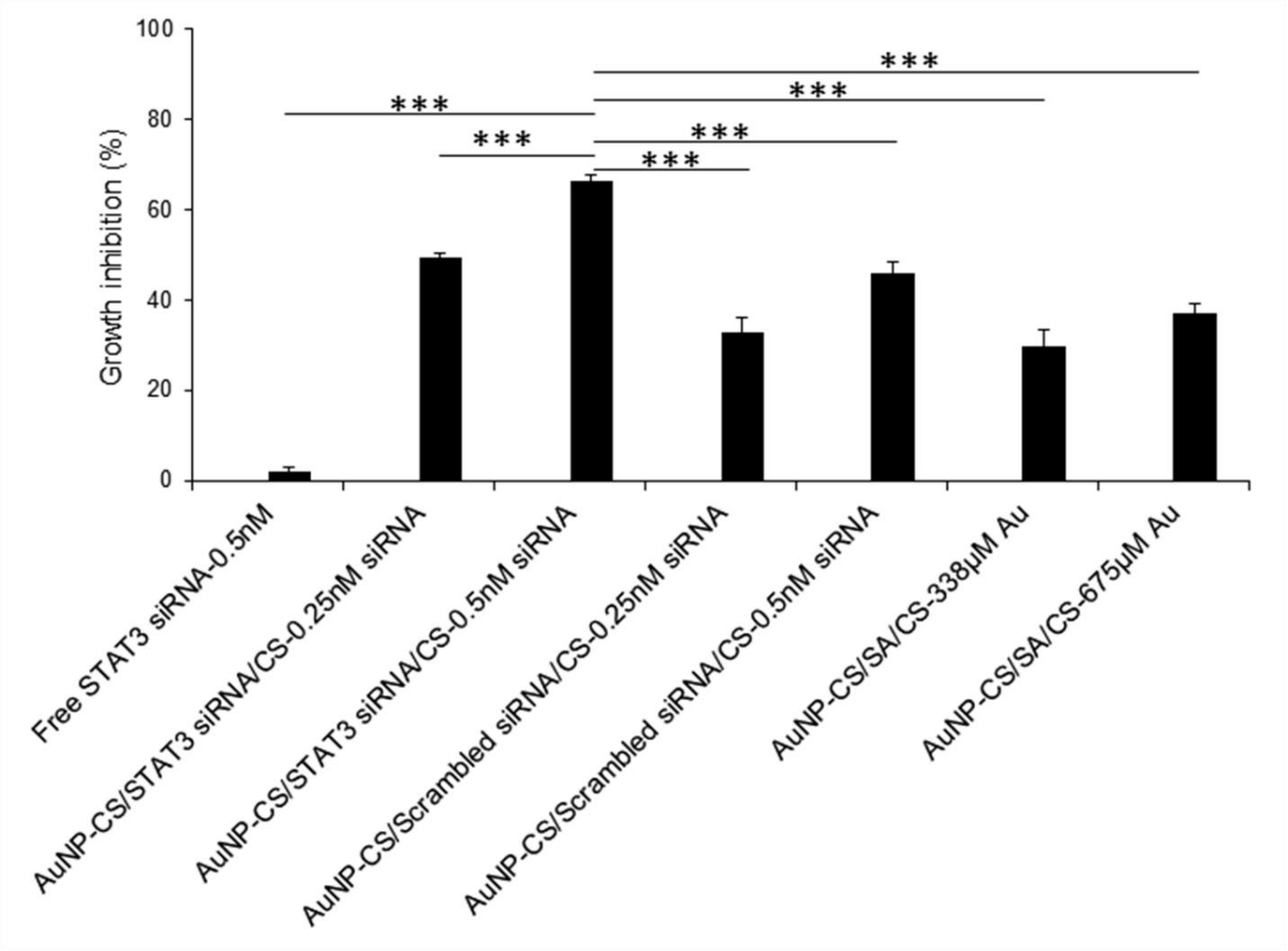




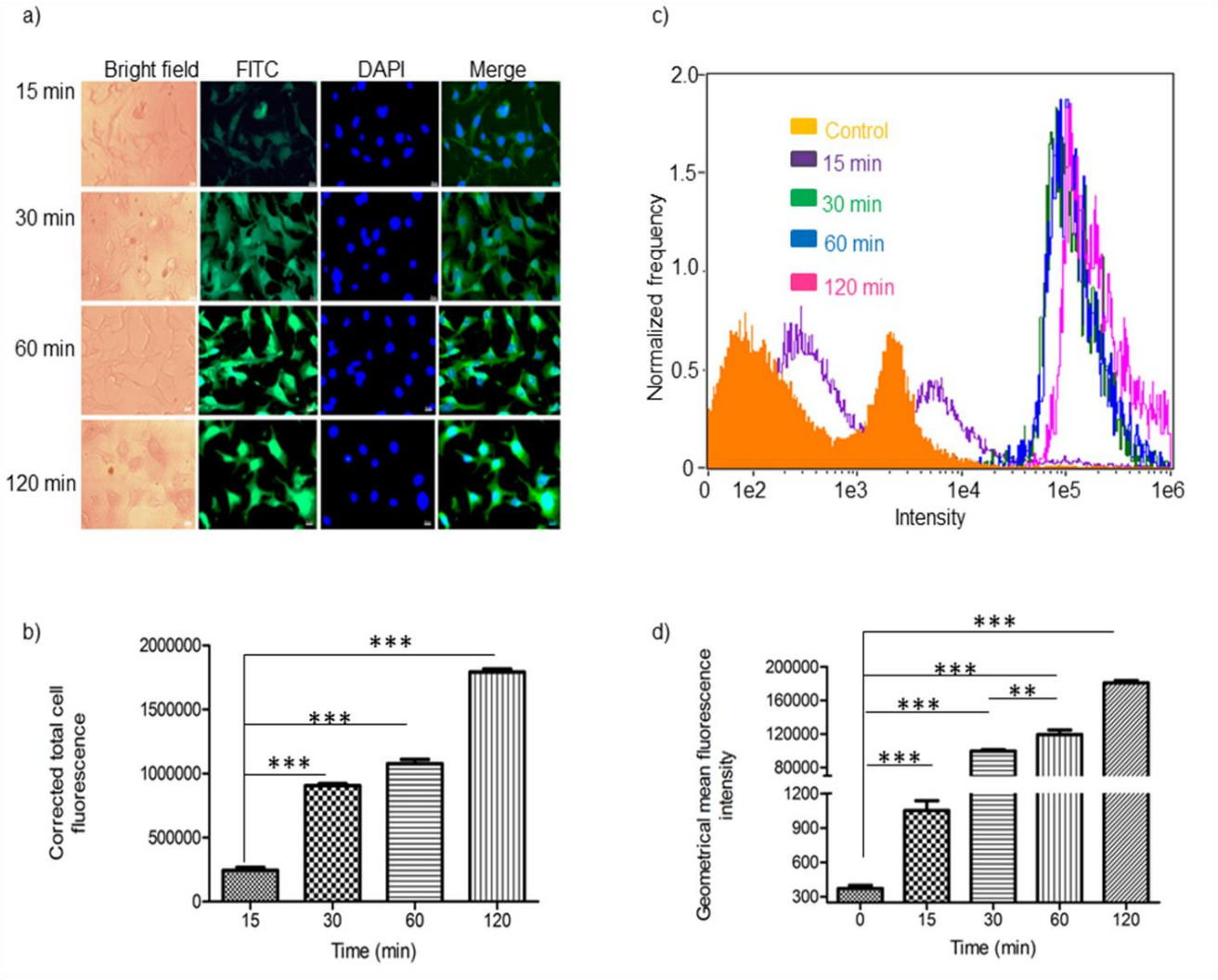




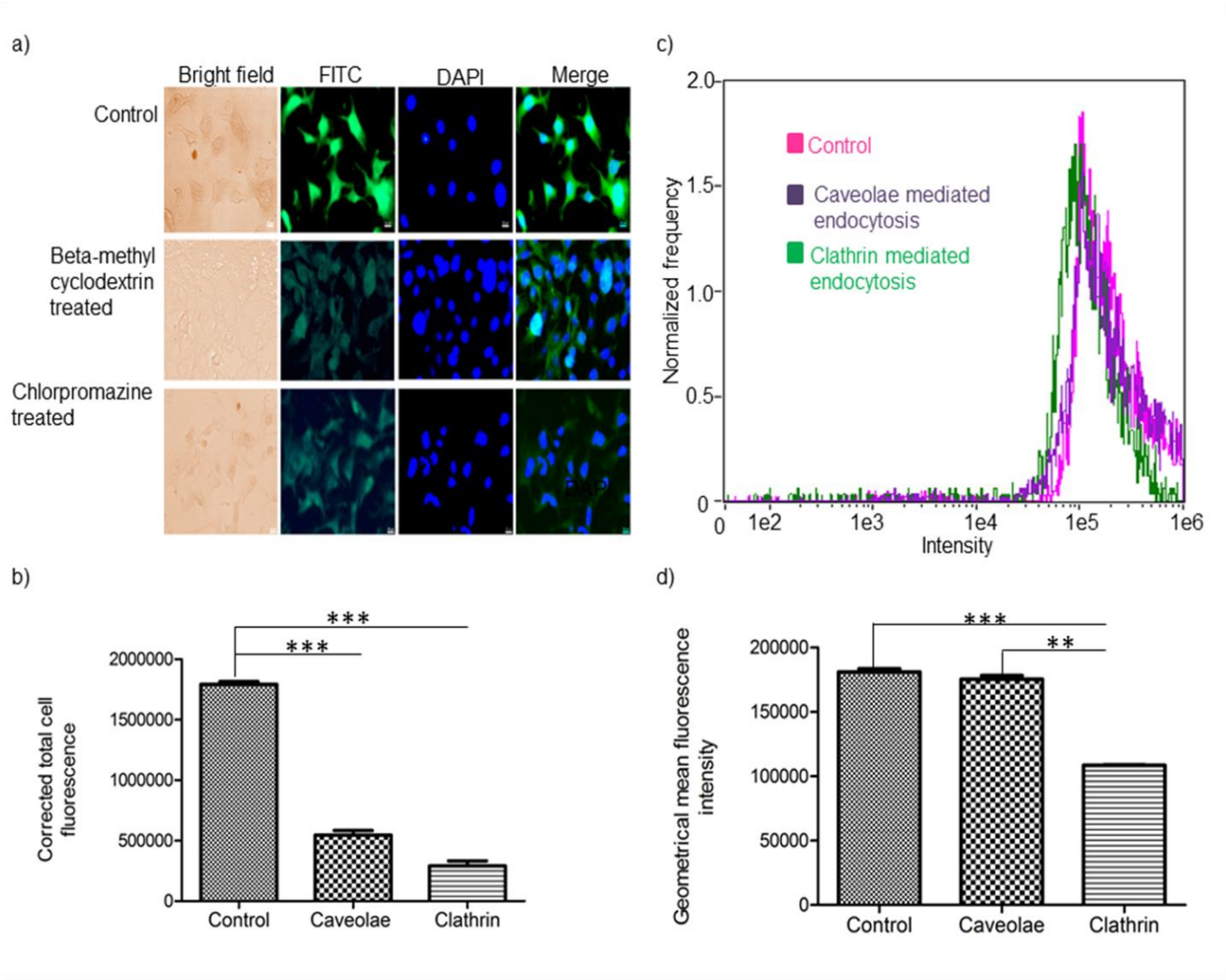
Cell viability study in B16F10



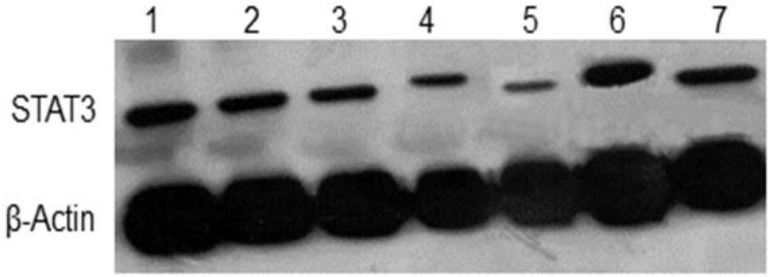
Cell uptake of LbL-AuNP



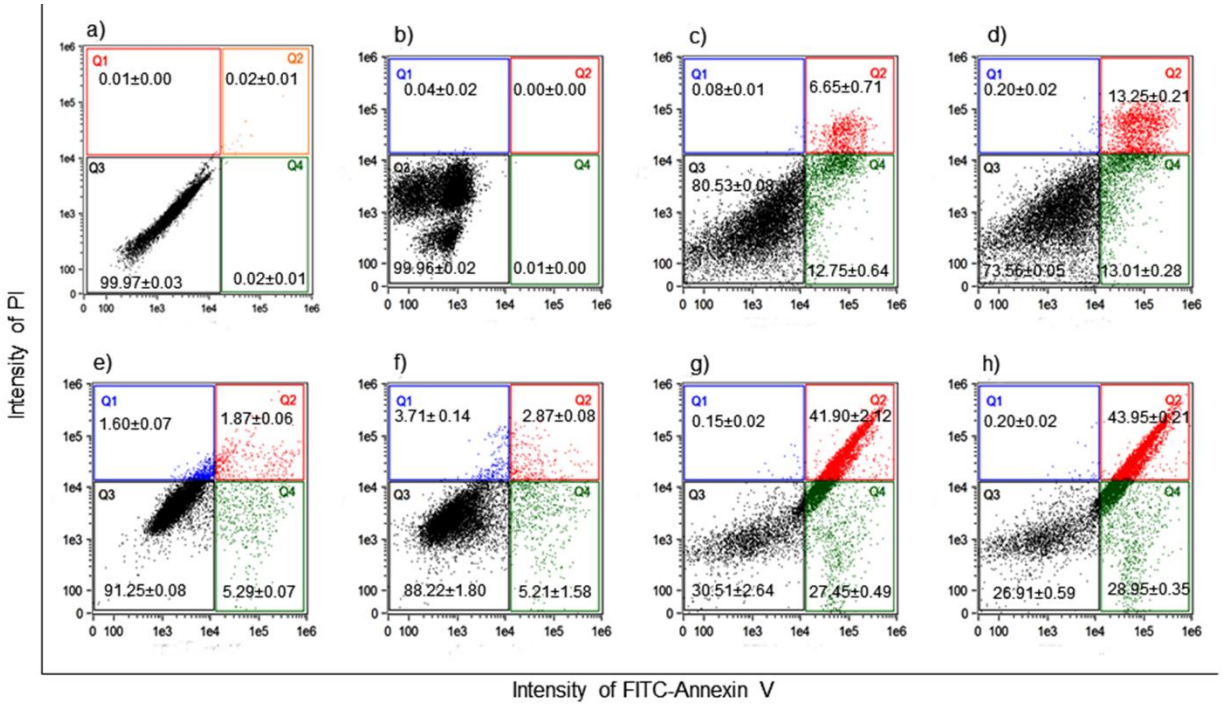
Mechanism of cell uptake



STAT3 expression and apoptosis events in B16F10 cells

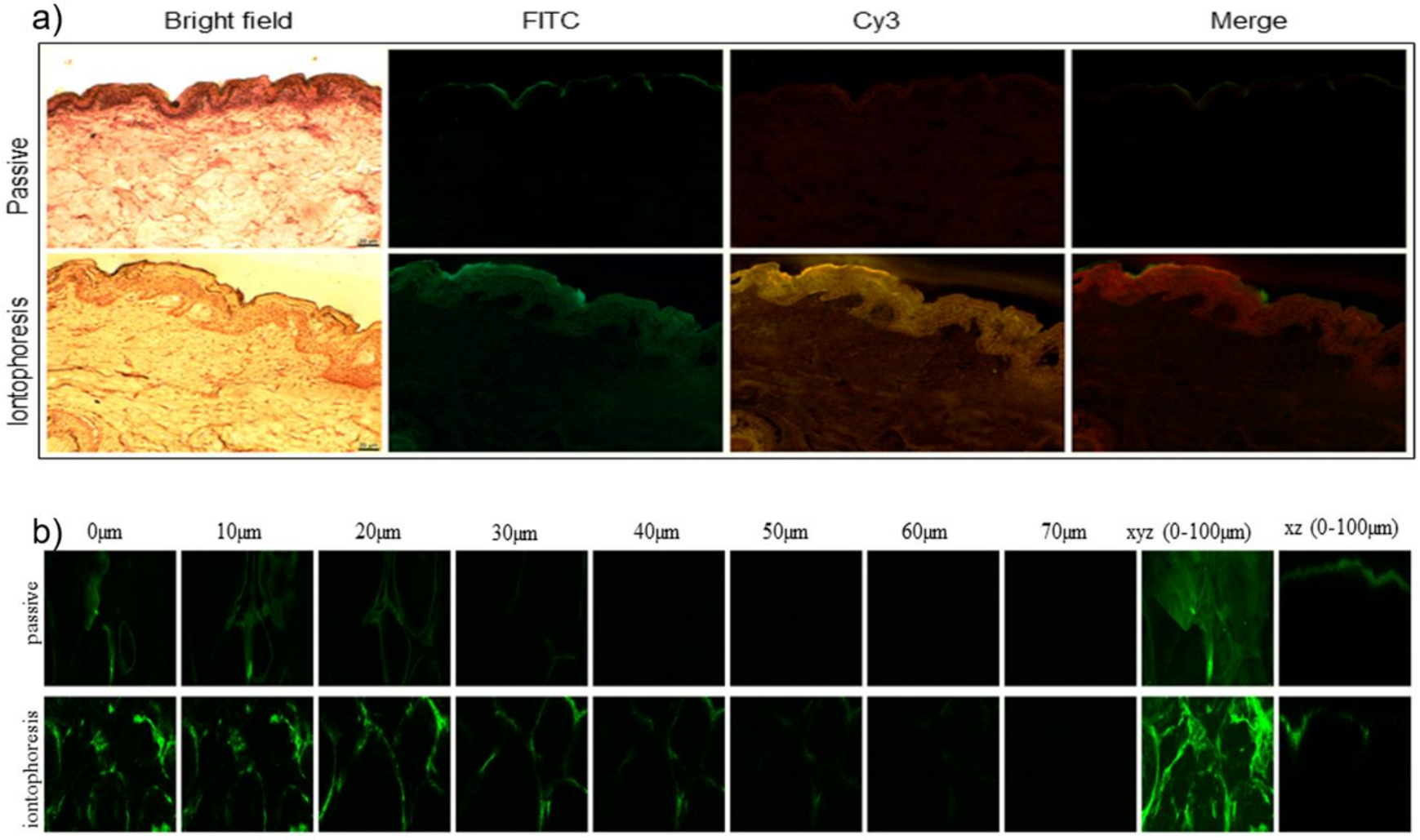


1. Untreated cells
2. AuNP-CS/Scrambled siRNA/CS containing 0.25 nM siRNA
3. AuNP-CS/Scrambled siRNA/CS containing 0.5 nM siRNA
4. AuNP-CS/STAT3 siRNA/CS containing 0.25 nM siRNA
5. AuNP-CS/STAT3 siRNA/CS containing 0.5 nM siRNA
6. AuNP-CS/SA/CS containing 338 µM of Au
7. AuNP-CS/SA/CS containing 675 µM of Au

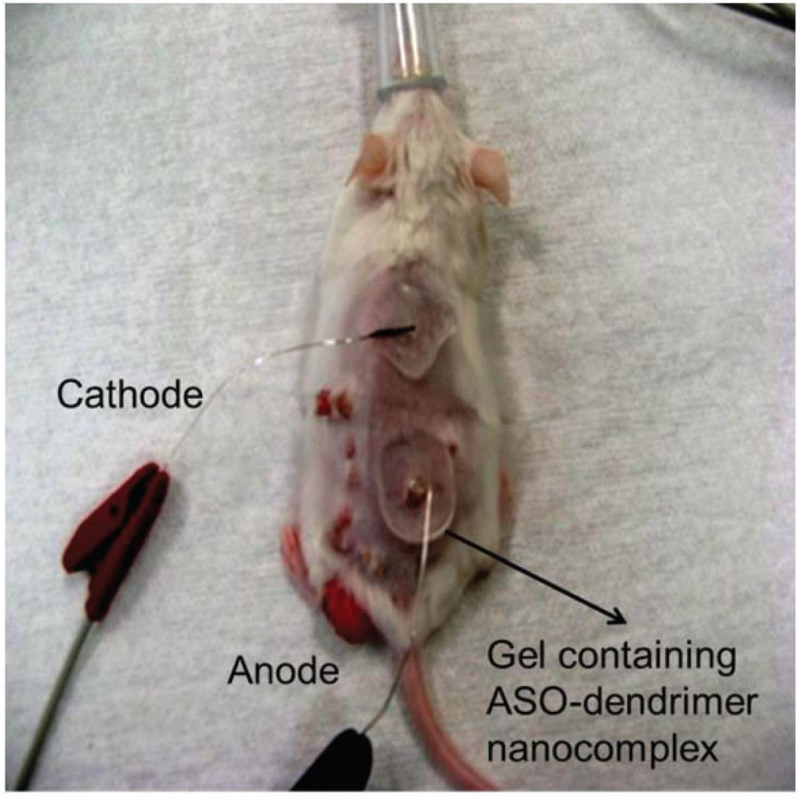


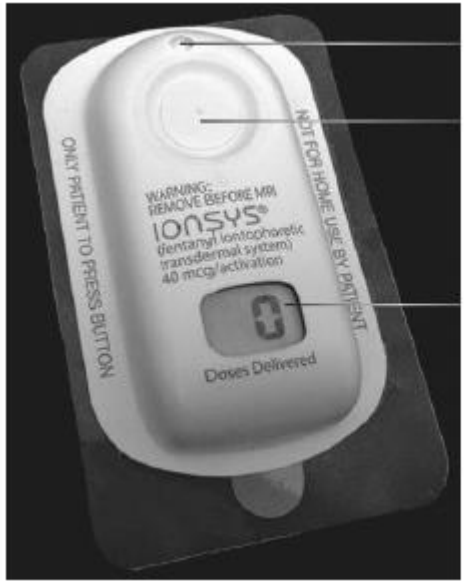
a, untreated cells; **b,** free STAT3 siRNA; **c & d,** AuNP-CS/SA/CS containing 338 µM and 675 µM of AuNP; **e & f,** AuNP-CS/Scrambled-siRNA/CS at 0.25 nM and 0.5 nM; **g & h,** AuNP-CS/STAT3-siRNA/CS at 0.25 nM and 0.5 nM, respectively

Skin penetration of siRNA loaded LbL-AuNP



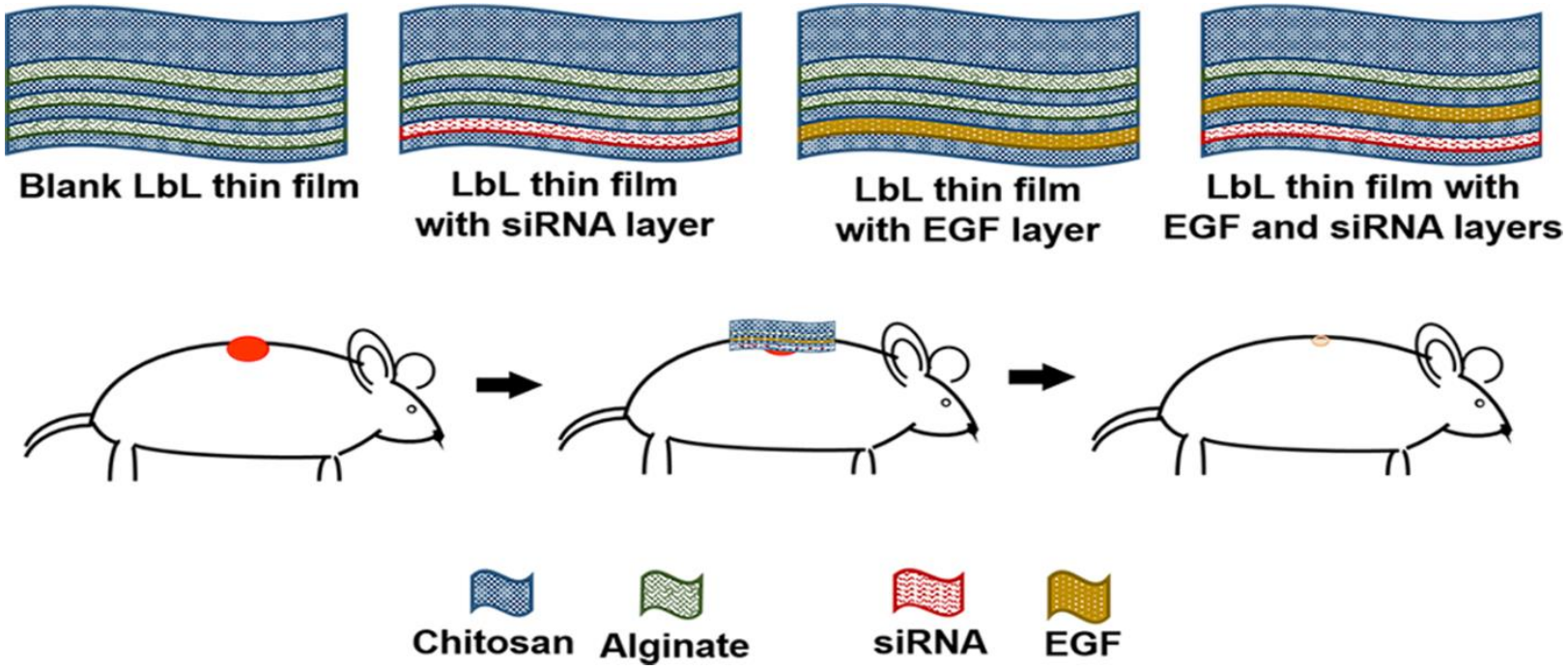
In-vivo application of iontophoresis



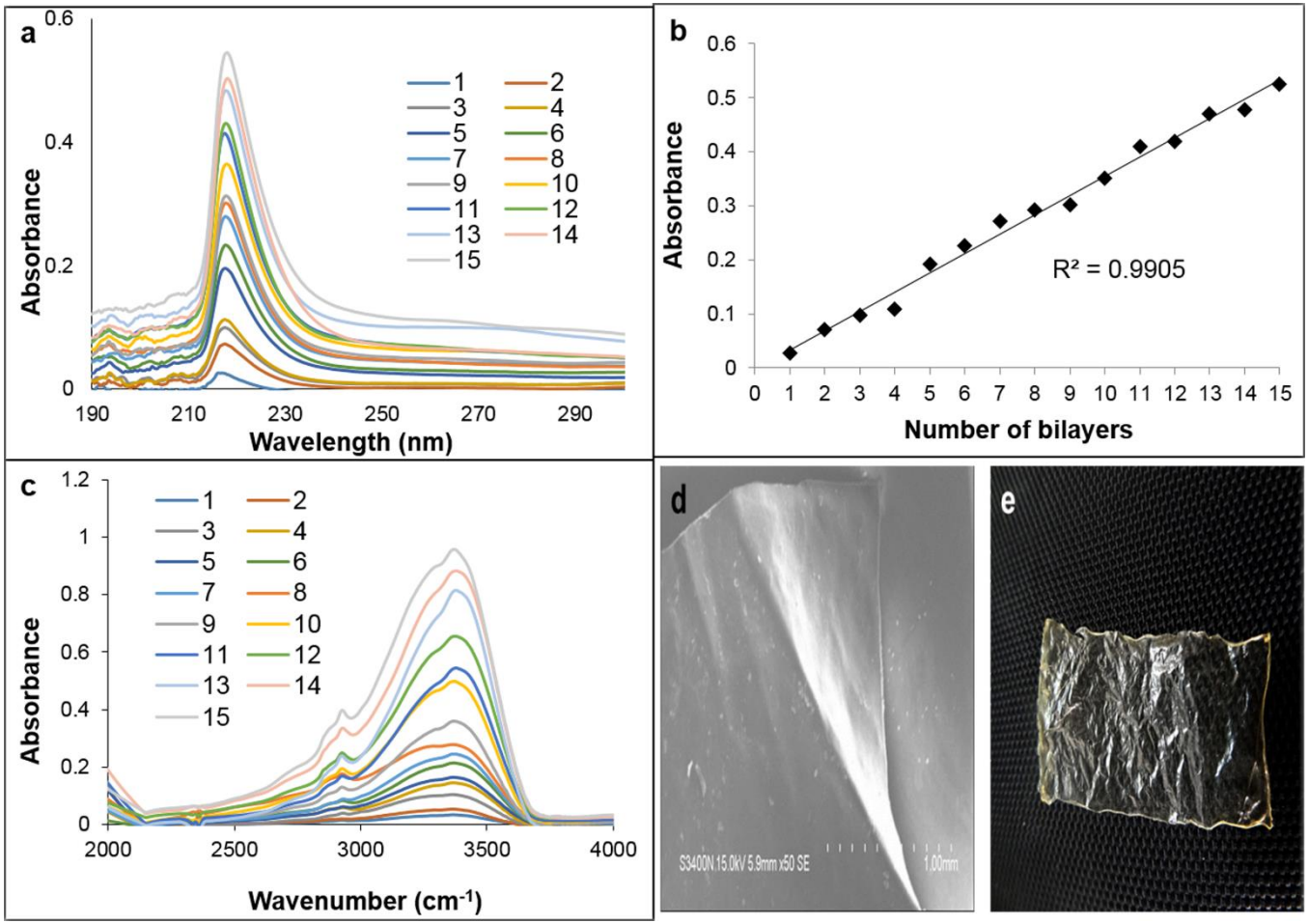


Venuganti et al. Nanoscale. 2015, 7(9):3903-14

2. Layer-by-layer thin films for topical applications

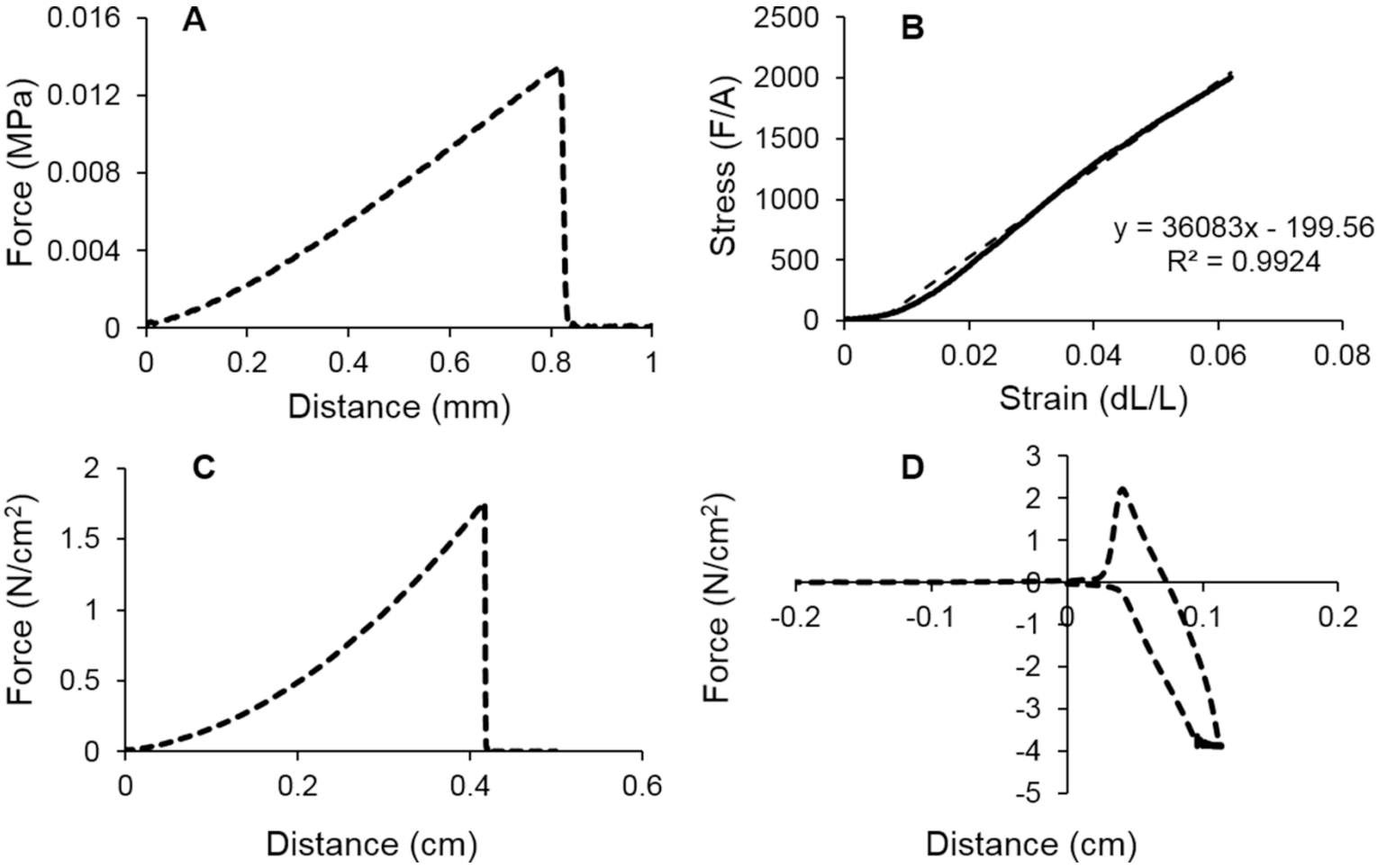


Characterization of LbL films



Mandapalli et al. Eur J Pharm Sci 83 (2016) 166–174

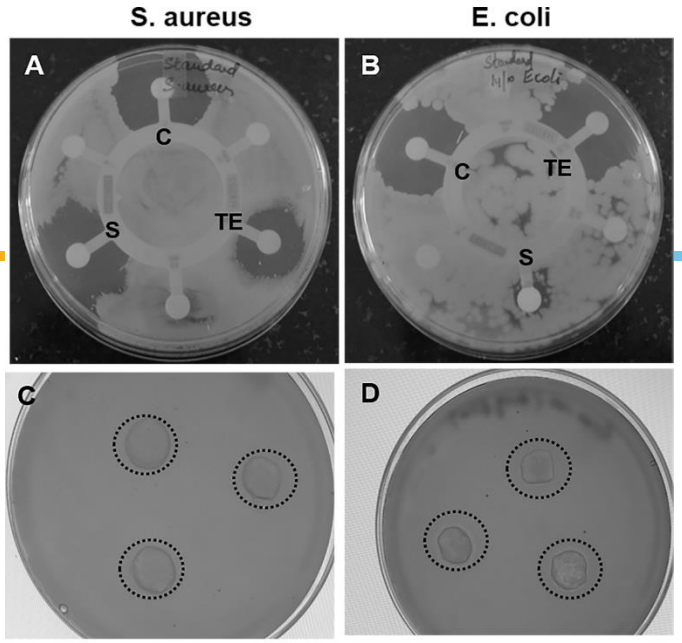
Physical characteristics of LbL blank films

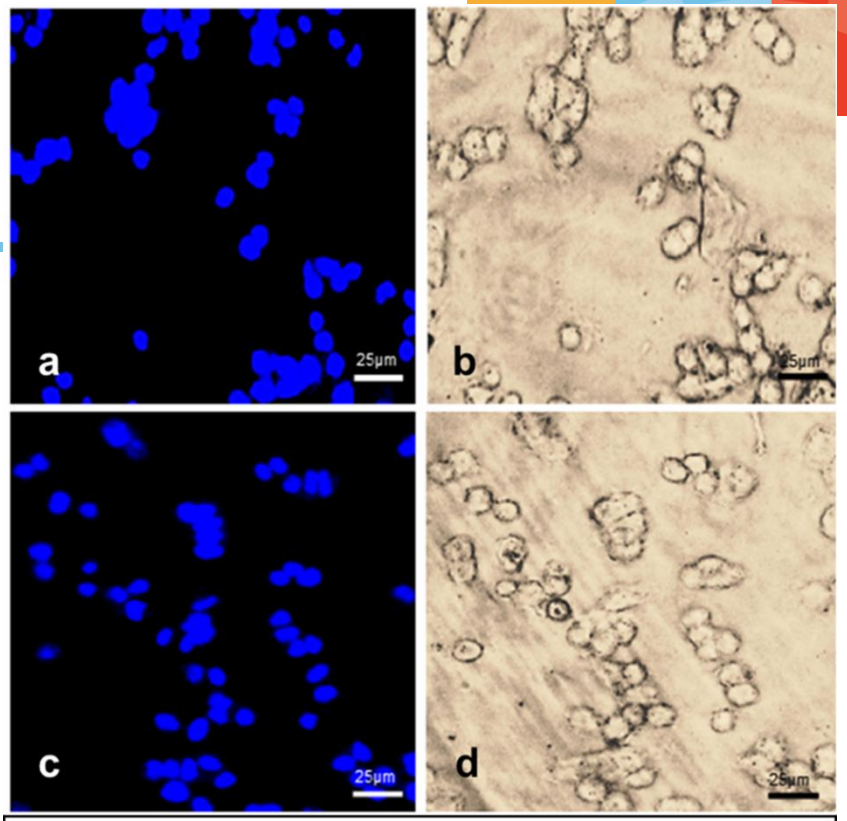


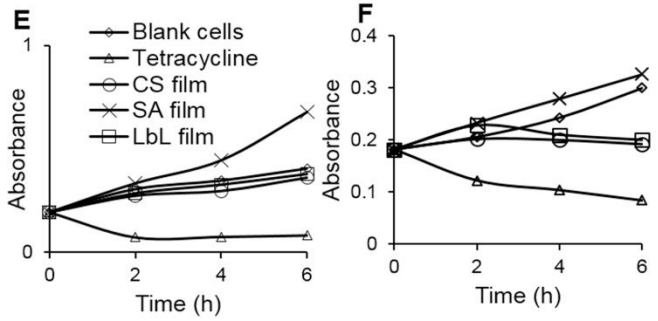
Physical characteristics of LbL blank films

Parameter	Value
Thickness (µm)	15 ± 1.0
Porosity (%)	27 ± 5.2
Swelling index (%)	32 ± 3.2
Burst strength (N/cm^2)	1.1 ± 0.09
Tensile strength (MPa)	0.013 ± 0.001
Elastic modulus (N/cm^2)	39.6 ± 3.2
Elongation (%)	3.56 ± 0.2
Adhesion strength (N/cm^2)	2.22 ± 0.08
Work of adhesion (N/cm^2)	0.37 ± 0.04

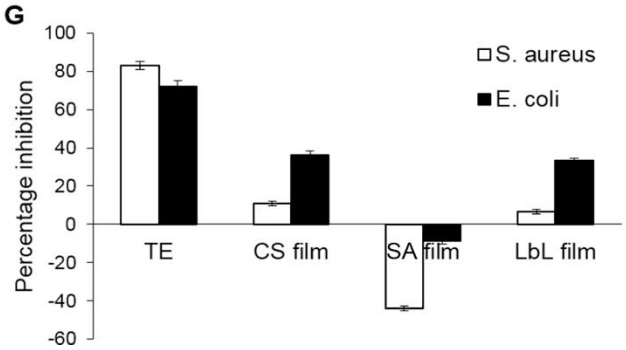
Data are presented as mean (n = 3) ± SD.


S. aureus
E. coli
A
B
C
TE
S
C
D

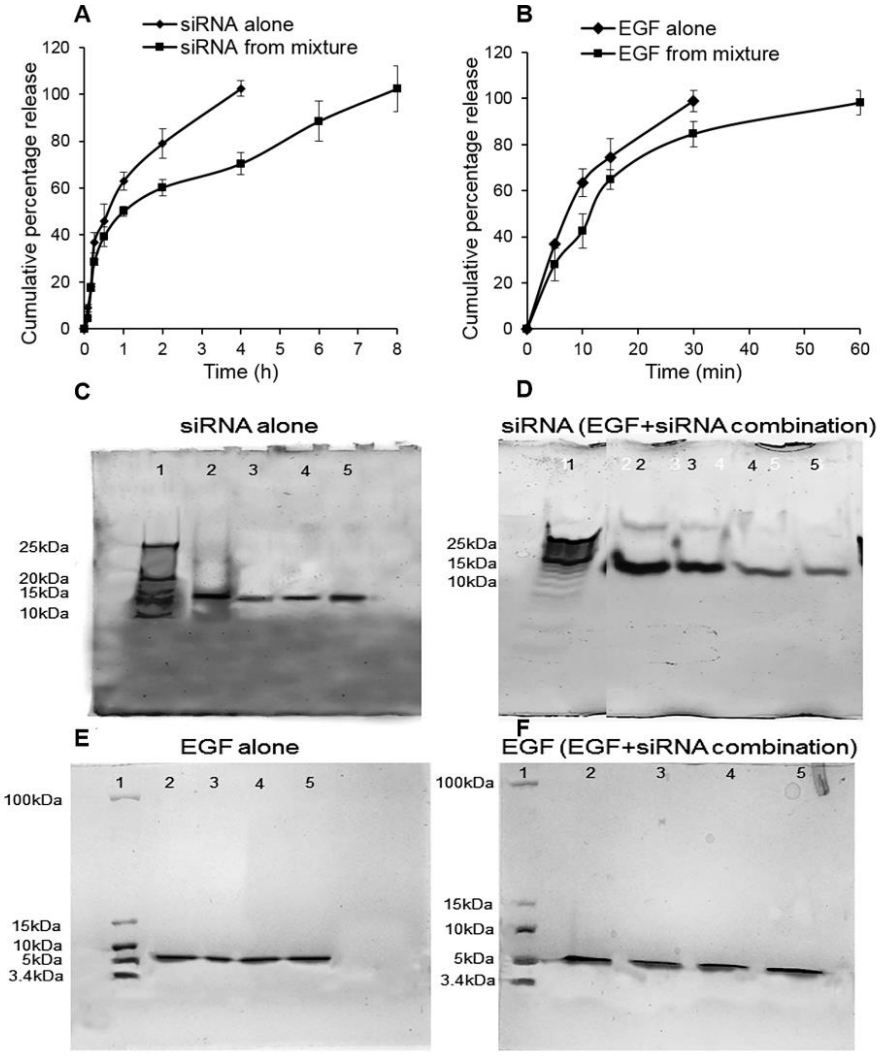

a
b
c
d
25μm


E
Blank cells
Tetracycline
CS film
SA film
LbL film
Absorbance
Time (h)
F
Absorbance
Time (h)

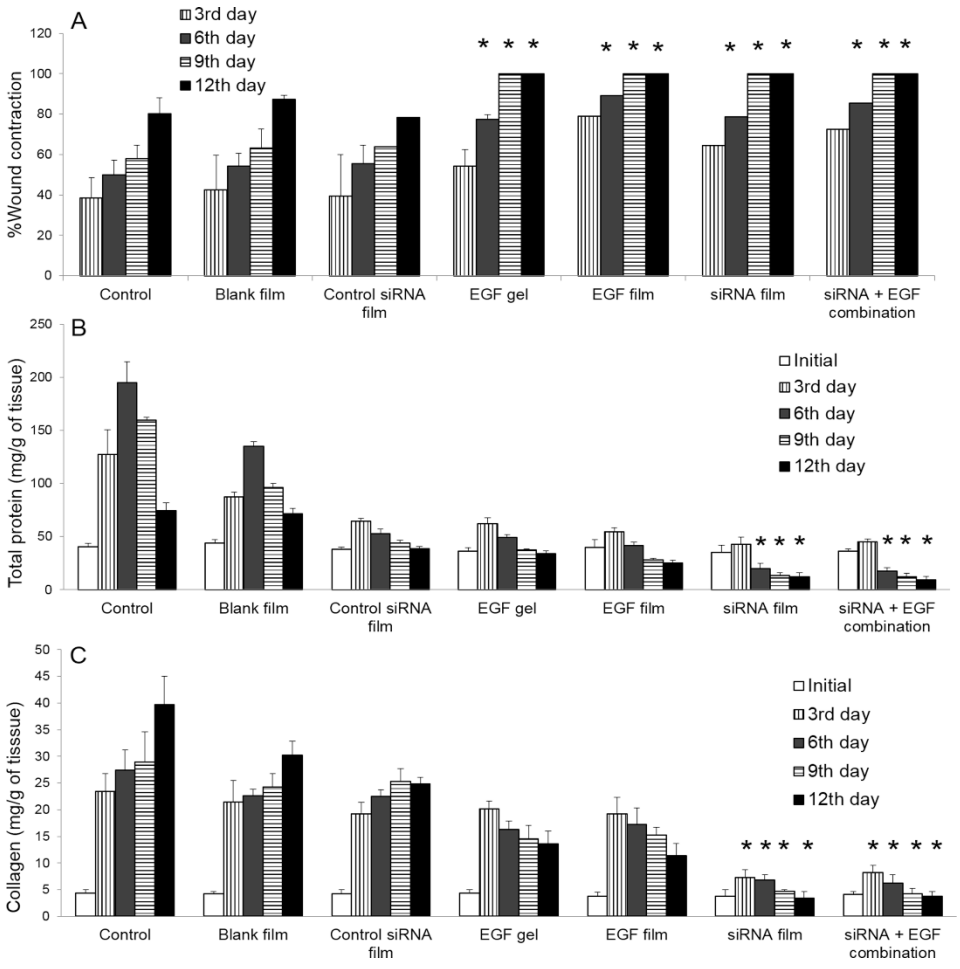

e
Cell viability (%)
Cultured in well plate
Cultured in LbL film


G
Percentage inhibition
S. aureus
E. coli
TE
CS film
SA film
LbL film

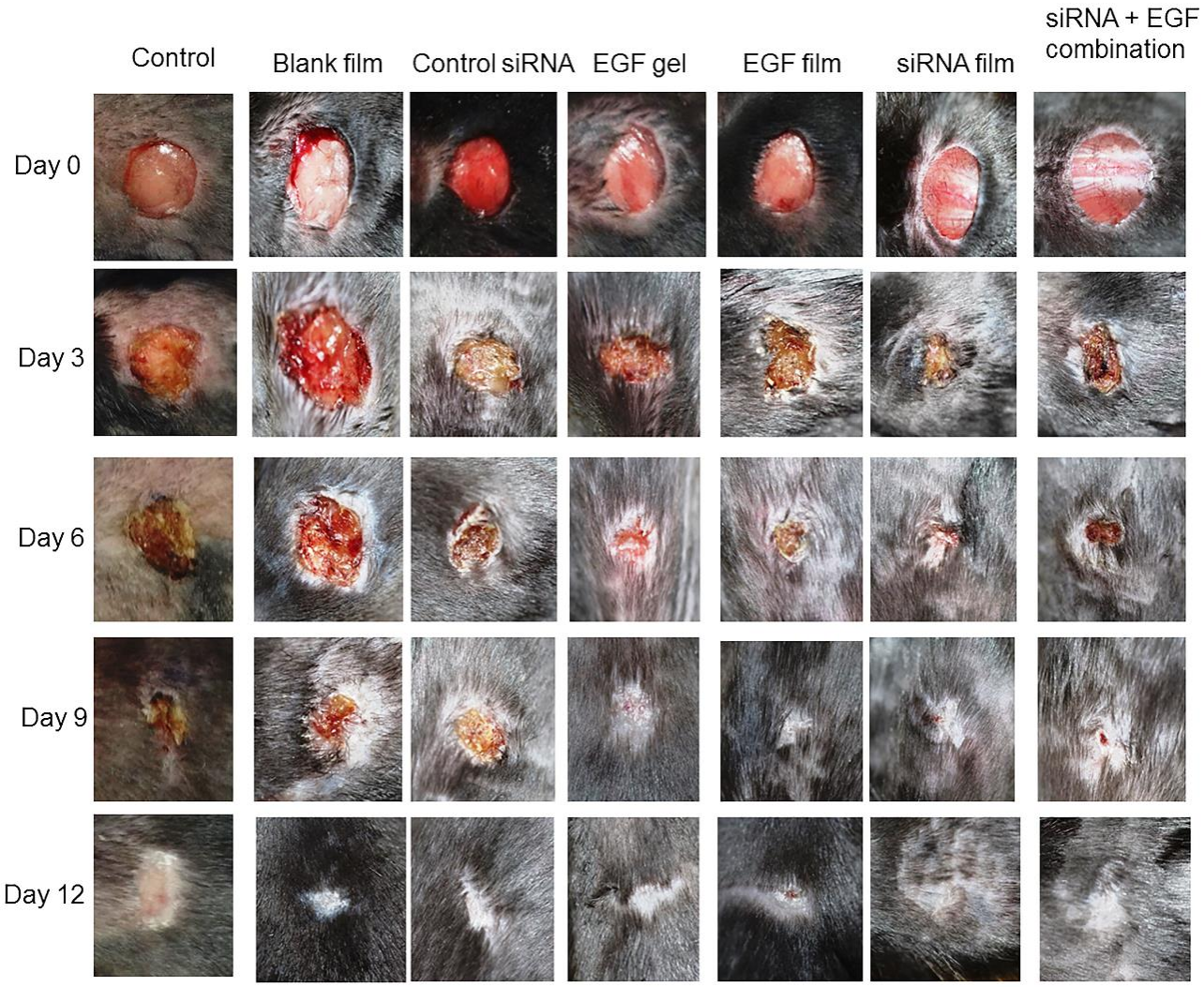
Characterization of siRNA loaded LbL films



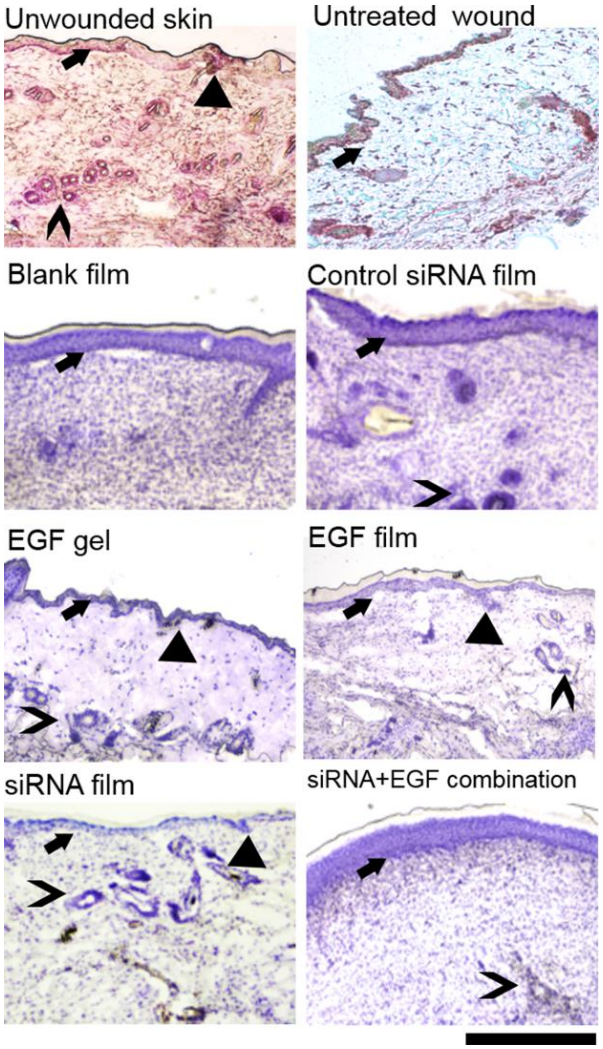
Excisional wound healing studies

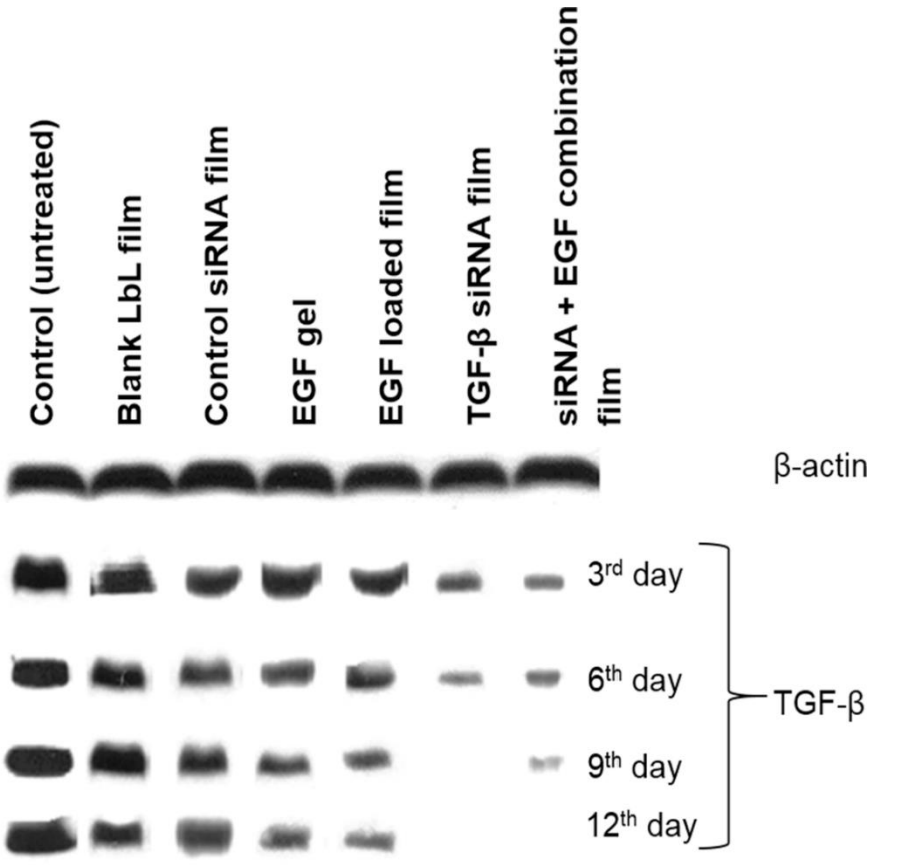


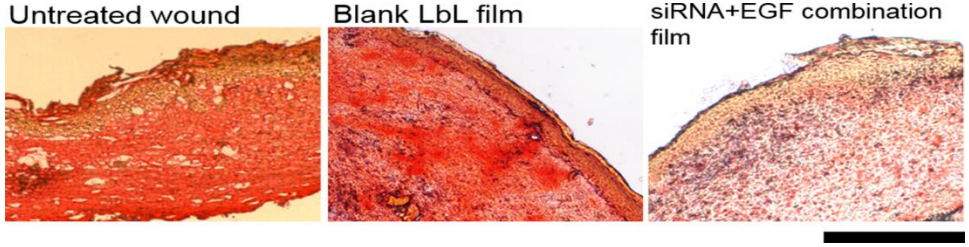
Excisional wound healing studies



Histological examination of wounded skin







Conclusions

- Layer-by-layer polyelectrolyte coating on AuNP would improve their stability in bio-relevant medium.
- High charge density on the LbL-AuNP would allow rapid cell uptake and allow iontophoresis application for skin transport.
- LbL thin films can be developed to loaded small and macromolecule drugs for topical applications on compromised skin.

Thank You!

Collaborators

Post-doctoral Research Associates
- Dr. G. Sudeep Kumar
- Dr. E. Kumar

PhD Students
- M. Praveen Kumar
- Mr. Suman Labala
- Mr. Anup Jose
- Ms. Shubhmita Bhatnagar

Funding source
- Department of Science and Technology (DST)
- Department of Biotechnology (DBT)
- BITS Pilani

Smart Self-healable Hydrogel based on Polymer Nanocomposite for Bio-medical Application

Prof. Nikhil K. Singha
Rubber Technology Centre
I.I.T. Kharagpur
INDIA

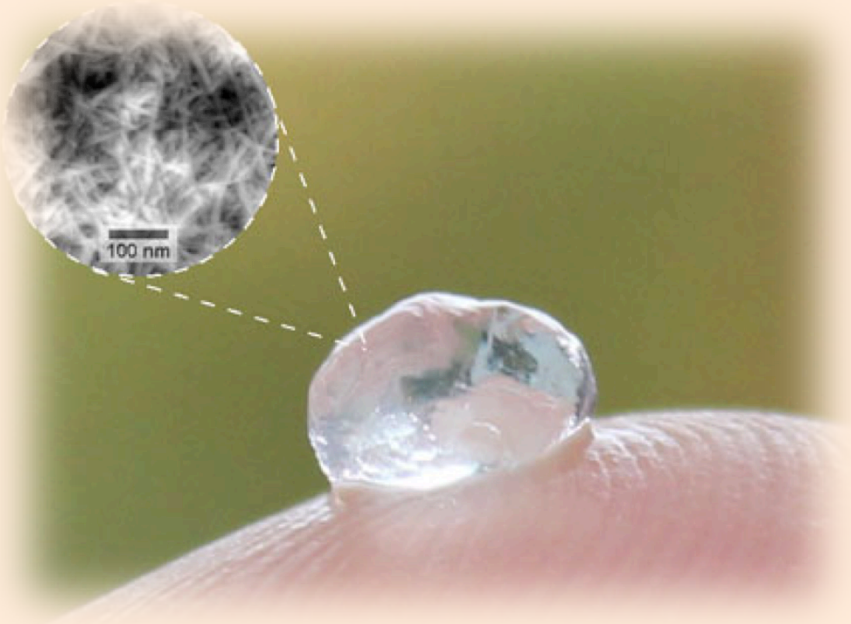
DBT-MRC Workshop March 14-15, 2016

Research Program in My Centre/ Research Group

- **Design of polymeric/rubber products**
- **Polymer blends**
- **Controlled/Living Radical Polymerization (CRP)**
- **Synthesis of Functional Polymers, Block and graft copolymers**
- **Preparation of smart materials by CRP & "Click Chemistry"**
- **Polymers in Bio-medical Applications**
- **Adhesives, Paints & Coatings (UV Curable, Reversible)**
- **Polymer Nanocomposites via *in situ CRP***
- **Ionic liquids in polymers**
- **Polymer Nanocomposites**
- **Thermoplastic elastomers**
- **Polyurethane (incl. fire retardant) Synthesis**
- **EMI Shielding materials**

What is hydrogel ?

A hydrogel is a macromolecular polymer gel constructed of a network of crosslinked polymer chains. Hydrogels are synthesized from hydrophilic monomers by either chain or step growth, along with a functional crosslinker to promote network formation. A net-like structure along with void imperfections enhance the hydrogel's ability to absorb large amount of water via hydrogen bonding.

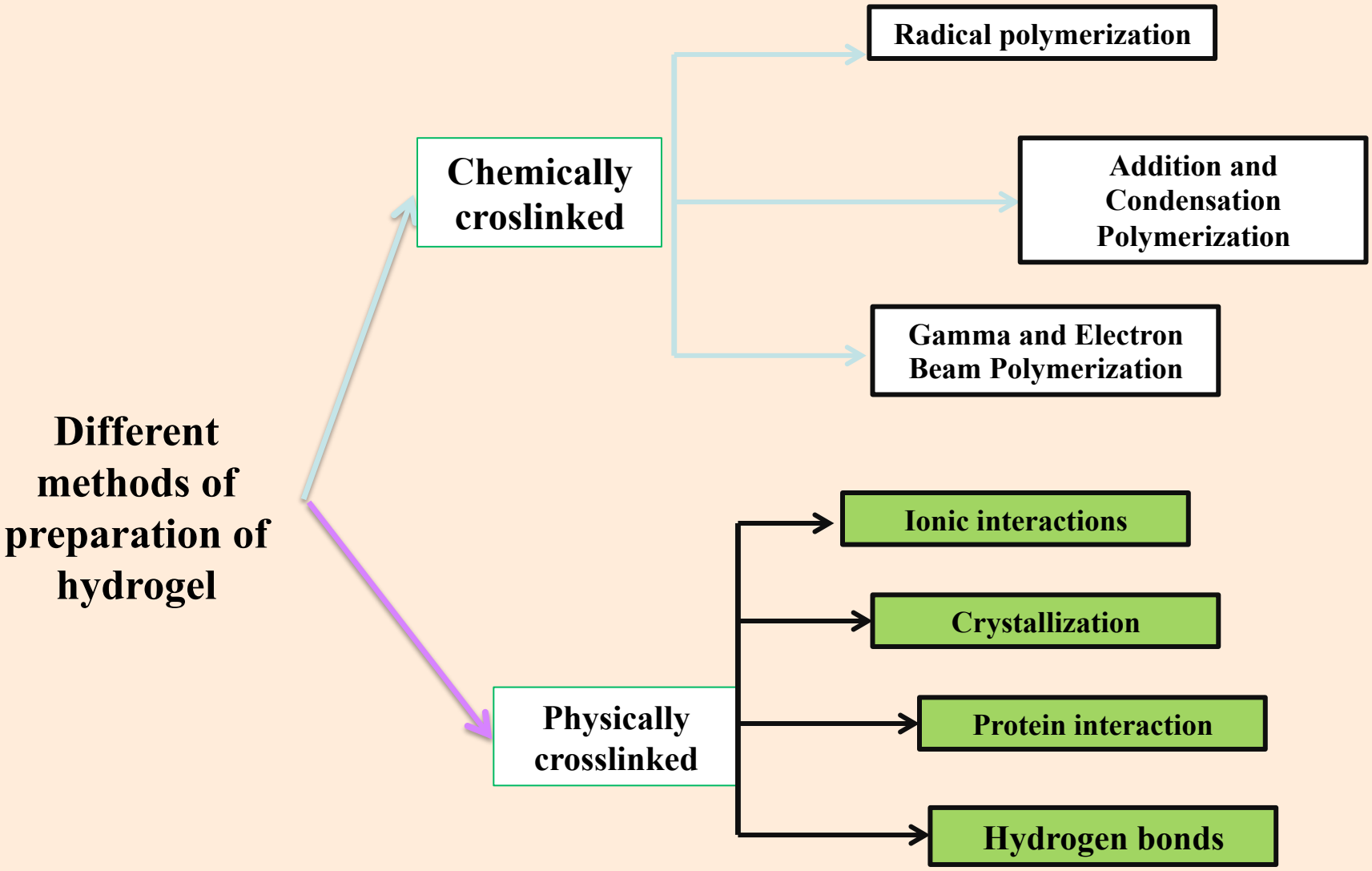


What is self healing hydrogel?

Self-healing refers to the spontaneous formation of new bonds when old bonds are broken within a material. The structure of the hydrogel along with electrostatic attraction forces drive new bond formation through reconstructive covalent dangling side chain or non-covalent hydrogen bonding. These flesh-like properties have motivated the research and development of self-healing hydrogels in fields such as reconstructive tissue engineering as scaffolding, as well as use in passive and preventive applications.

Viswanathan et.al. Nature, (2001), 409(6822), 794.

Synthesis methods of hydrogel

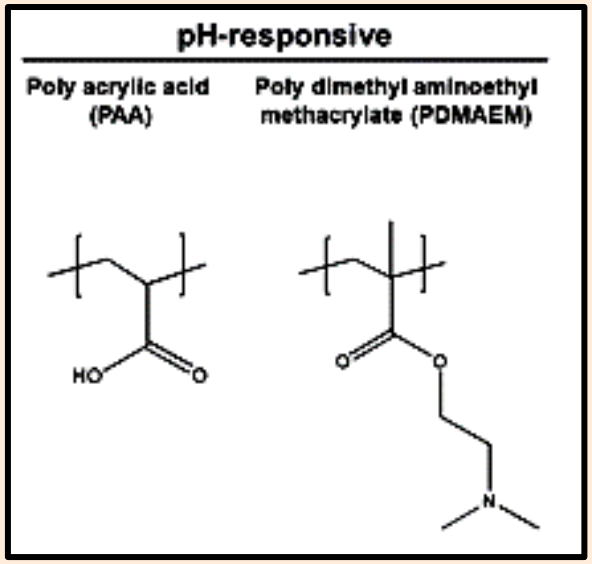


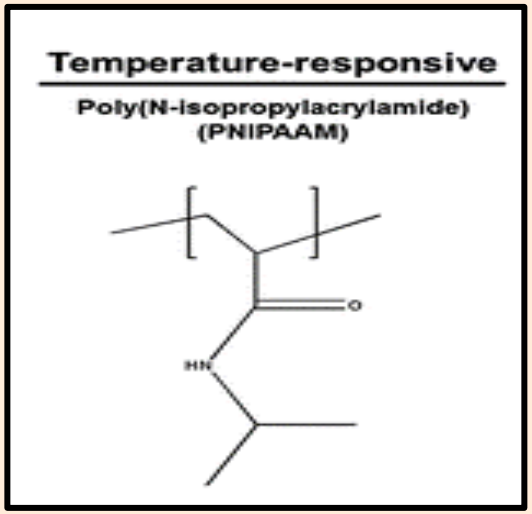
Stimuli responsive hydrogel

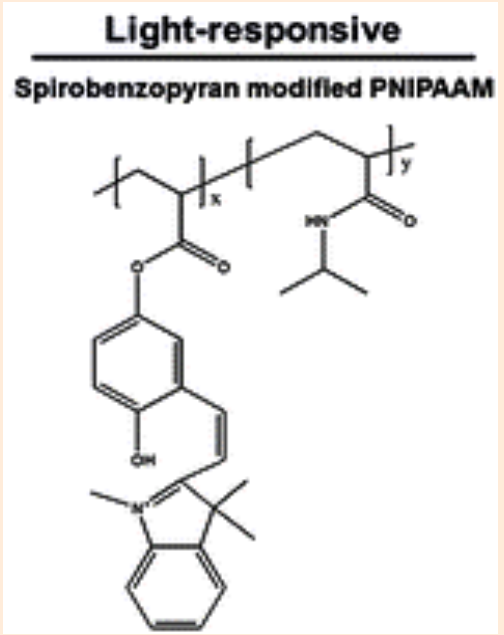
pH responsive

Thermo responsive

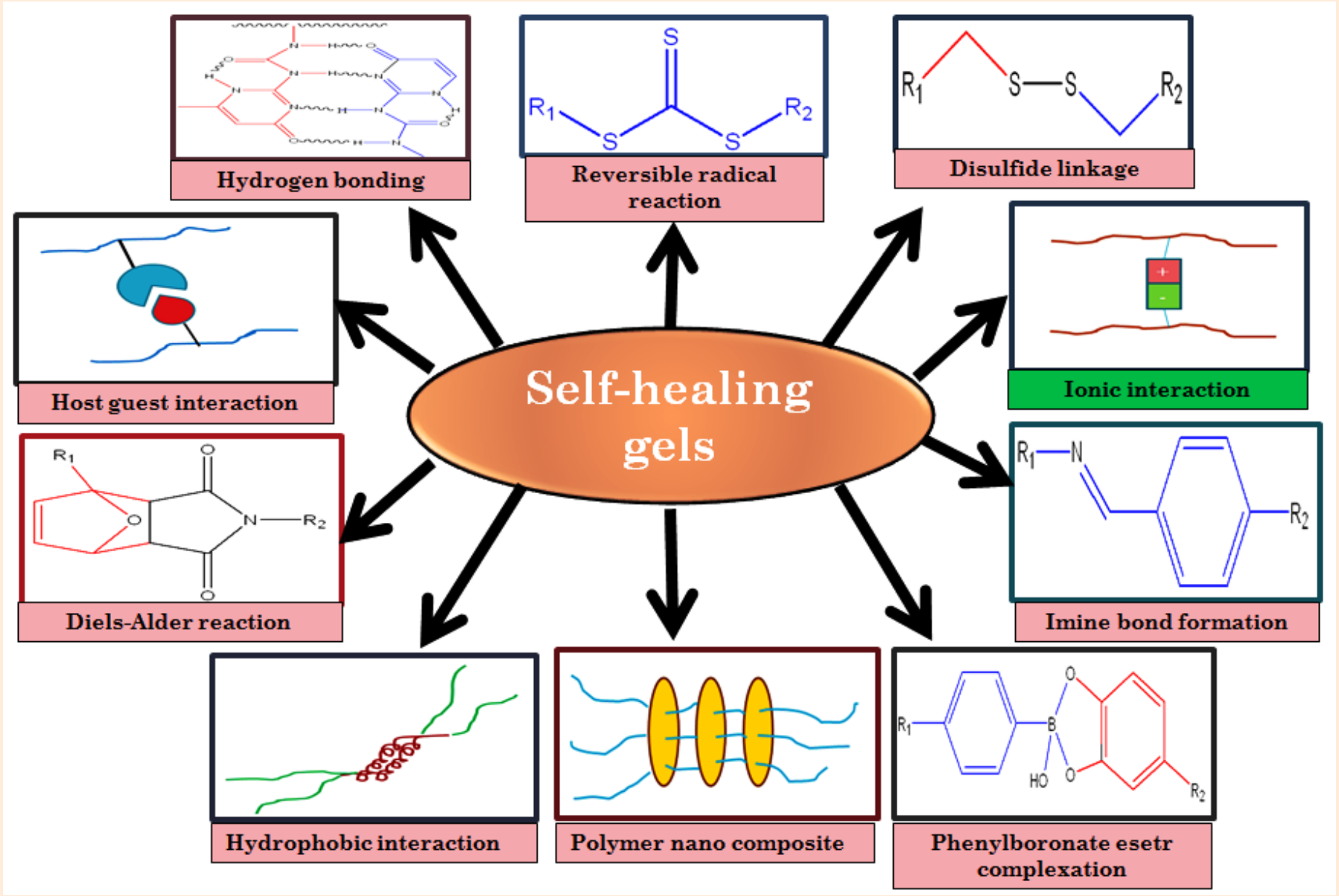
Light responsive





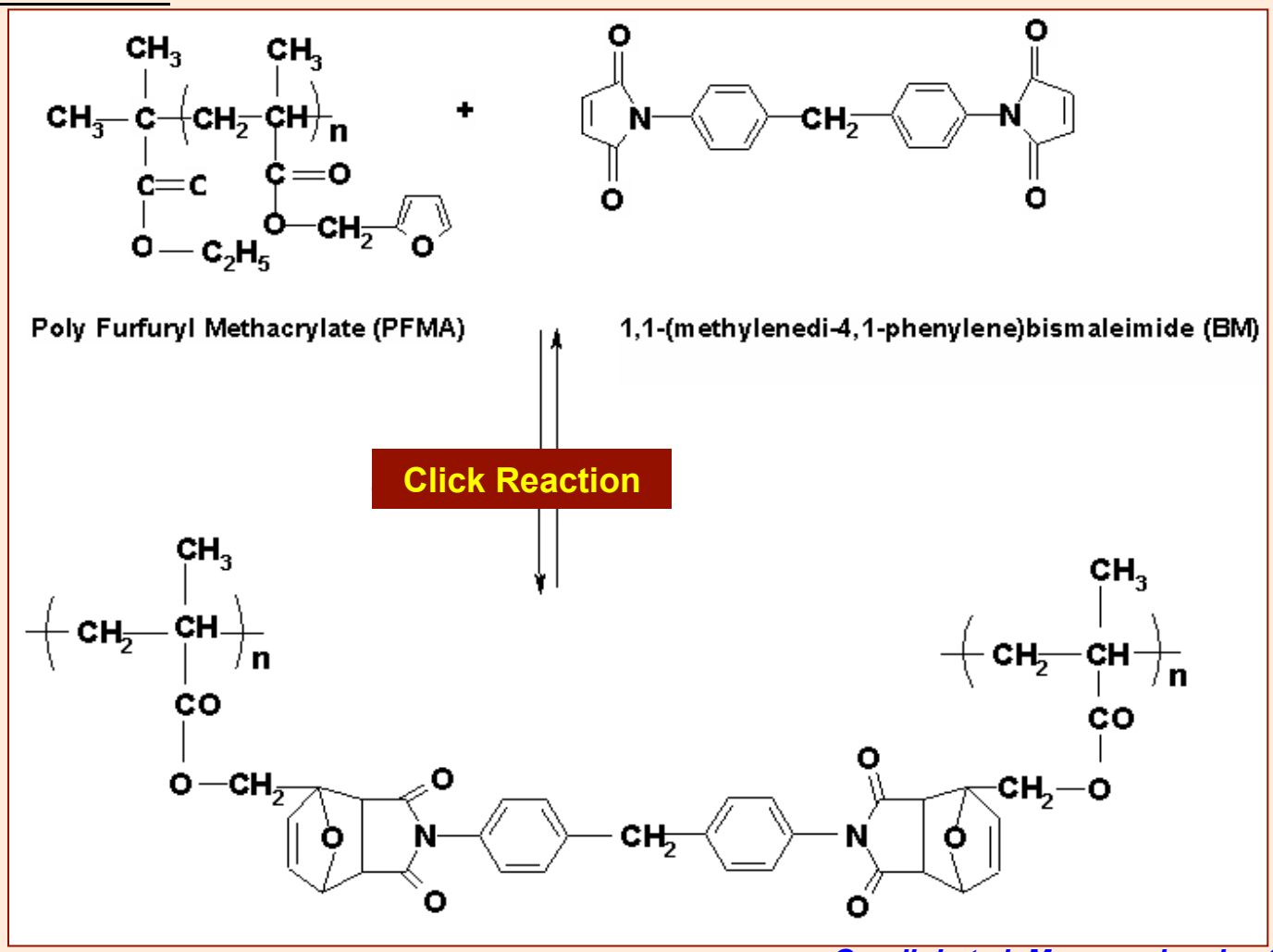


Different mechanisms of self healing in polymer gel



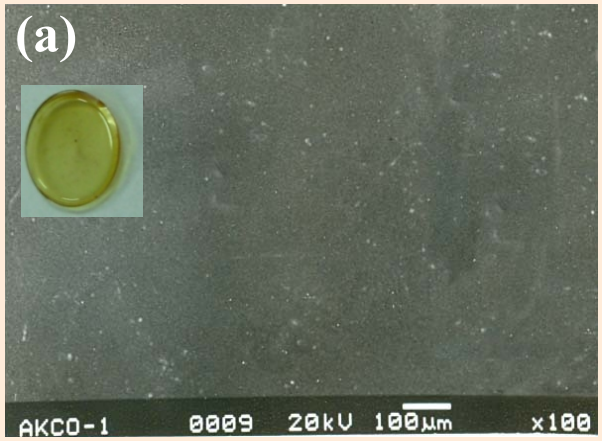
Diels Alder (DA) & rDA Reaction in Polyfurfuryl Methacrylate (PFMA)

Reaction Scheme:

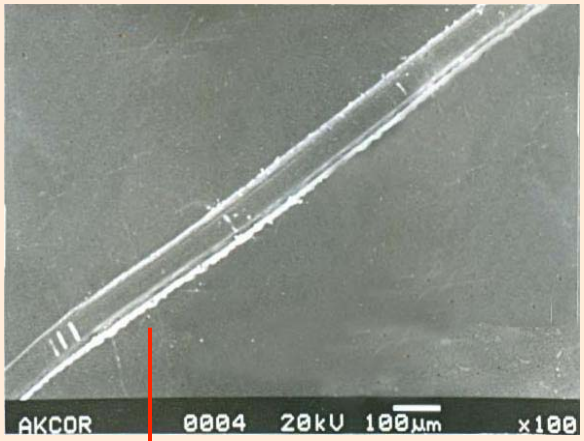


Gandini et al, Macromolecules 2002
Wudle et al Science 2002
Kavitha & Singha J. Polym. Sci. Polym. Chem. 2007
Kavitha & Singha Macromolecules 2010

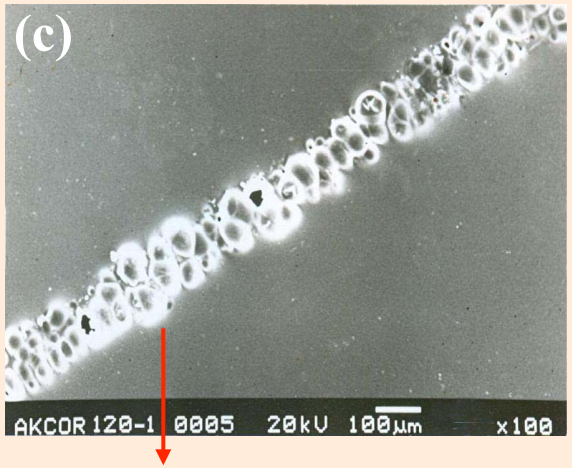
Thermo-reversible Self-healing DA Polymer



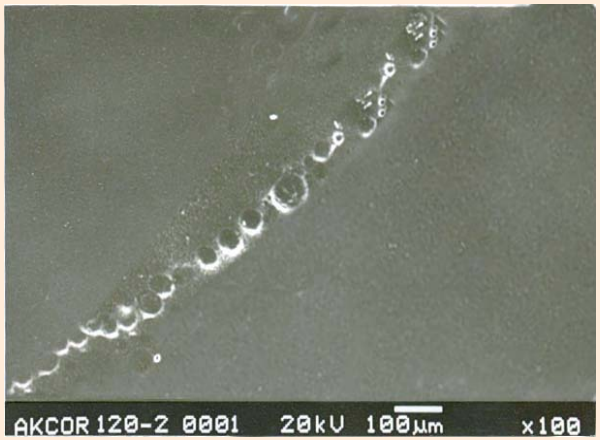
very smooth



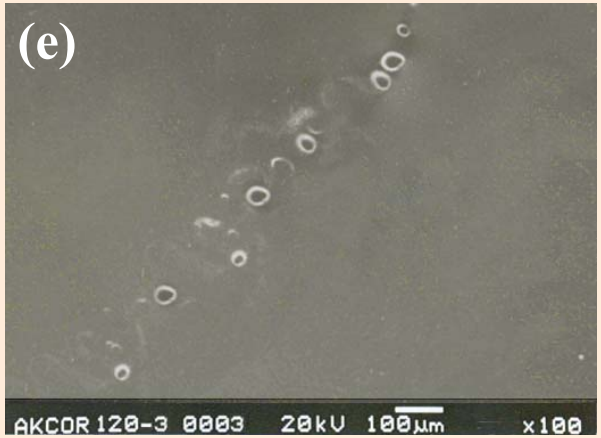
notch



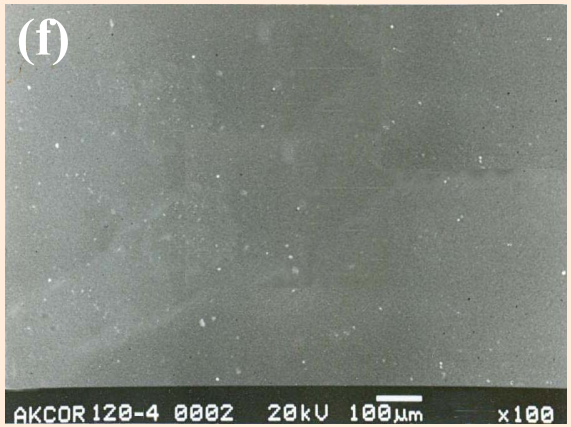
Heated at 120 °C (1 h)



2 h



3 h

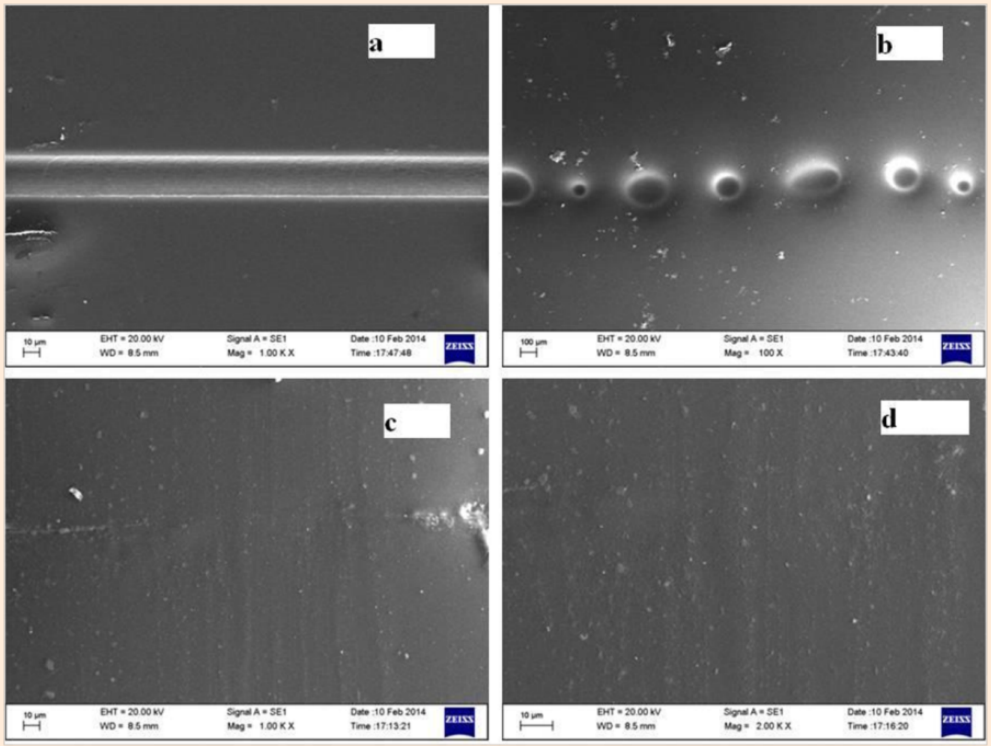


4 h

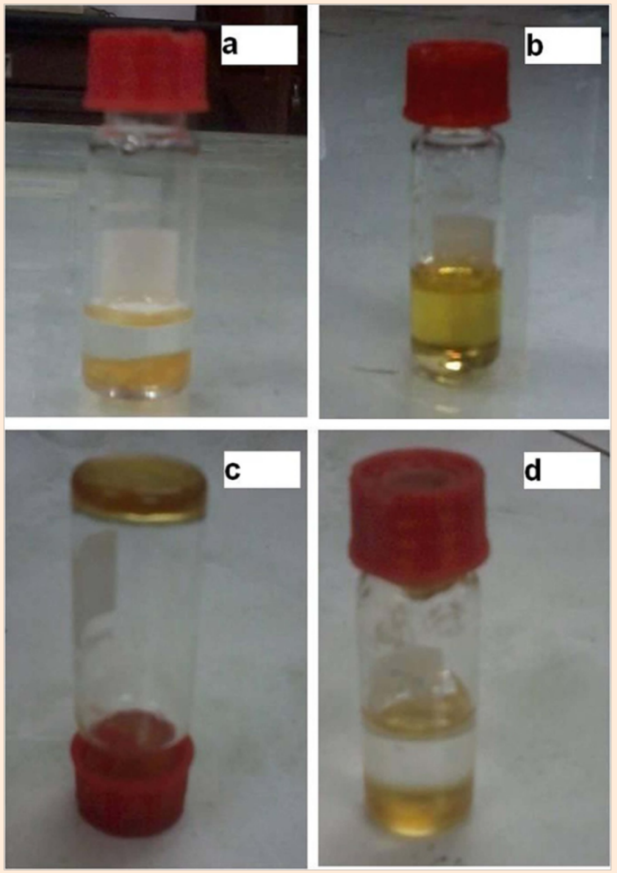
Self-repairing Properties of Polyfurfuryl methacrylate via FE-SEM analysis

Kavitha & Singha ACS Applied Materials & Interfaces, 1, 1427 (2009), *Macromolecules 2010*.

Healing & Thermoreversibility of Copolymer/BM adduct



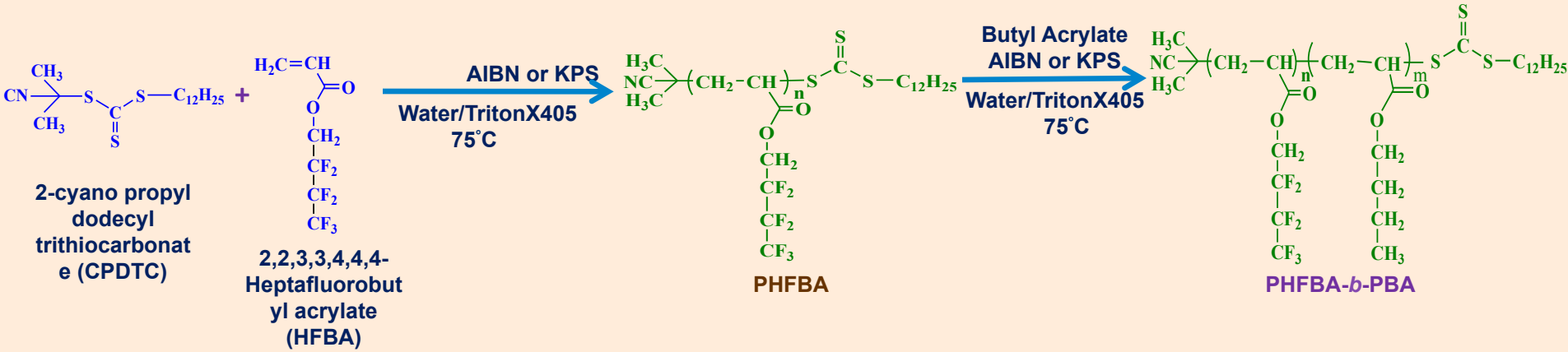
SEM images of healing of a notch on copolymer/BM DA adduct healed with time on heating & cooling followed by cooling at 50 °C for 24 hr. a) notch surface, b) 1 h, c) 2 h, d) 4 h.

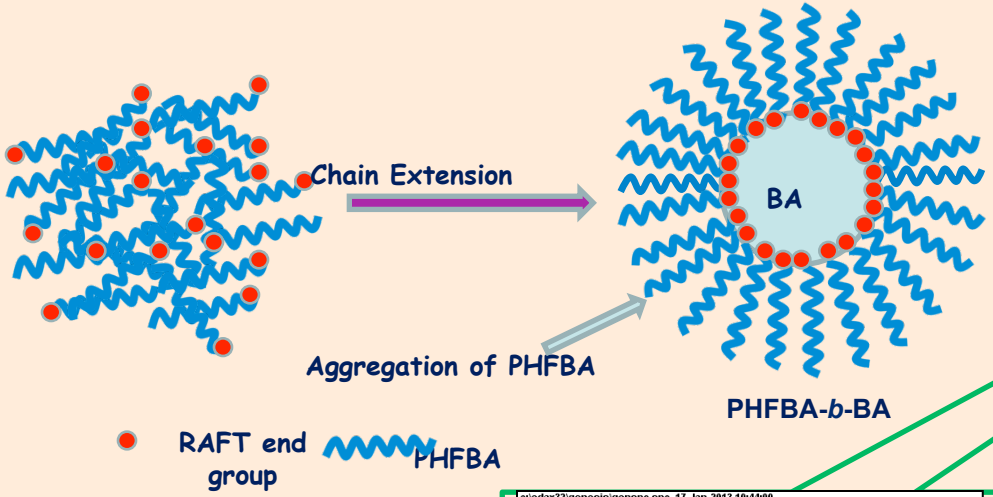


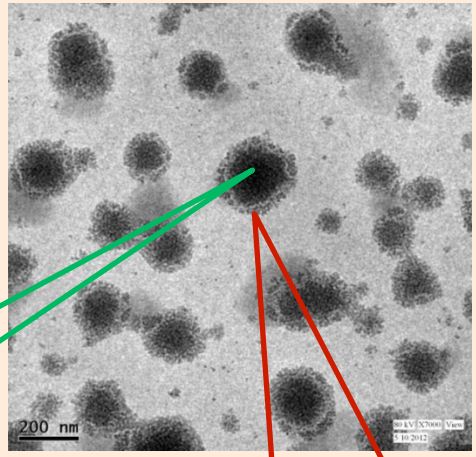
a) Cross-linked DA polymer (PFMA-co PBMA/BM) insoluble in toluene at 30 °C, b) soluble after heated at 120 °C, c) Reformed polymer was gelled when this soluble polymer was cooled, d) Insoluble polymer at 30 °C.

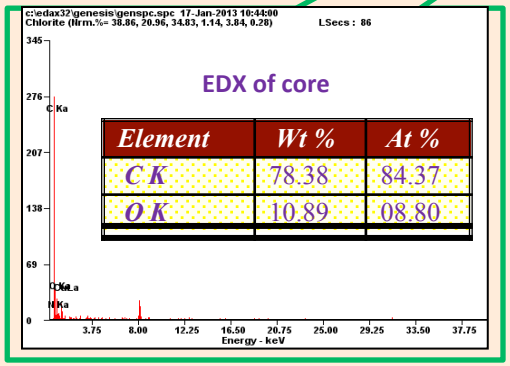
Polymer 2015

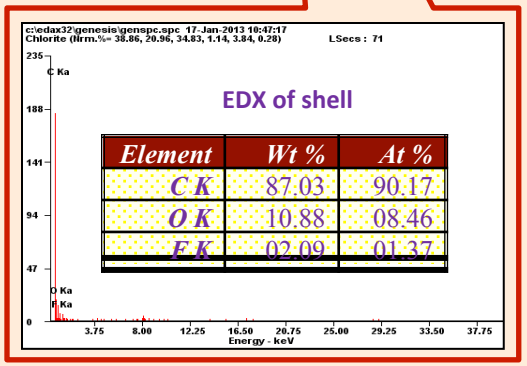
Core-shell fluorinated block copolymer With improved hydrophobicity





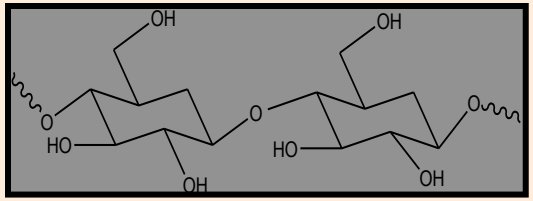




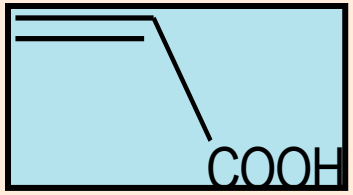


Chakrabarty & Singha;
J. Colloid. Interface Science 2013, 408, 66-74

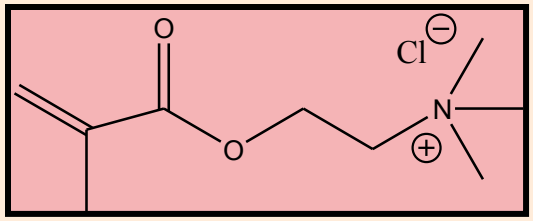
Materials used for hydrogel preparation



Starch (Str)

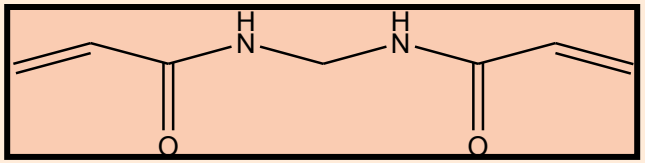


Acrylic acid (AA)

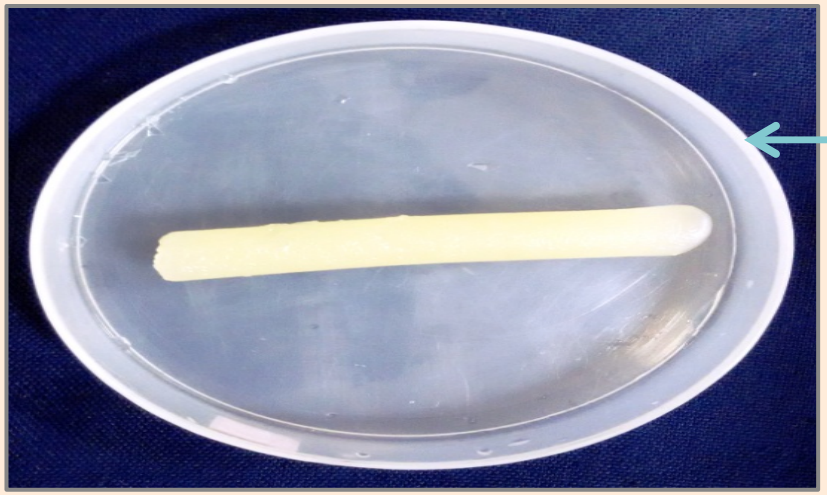


2-acryloyloxy)ethyl trimethyl ammonium chloride (AETAC)

Organically modified clay (OMMT)

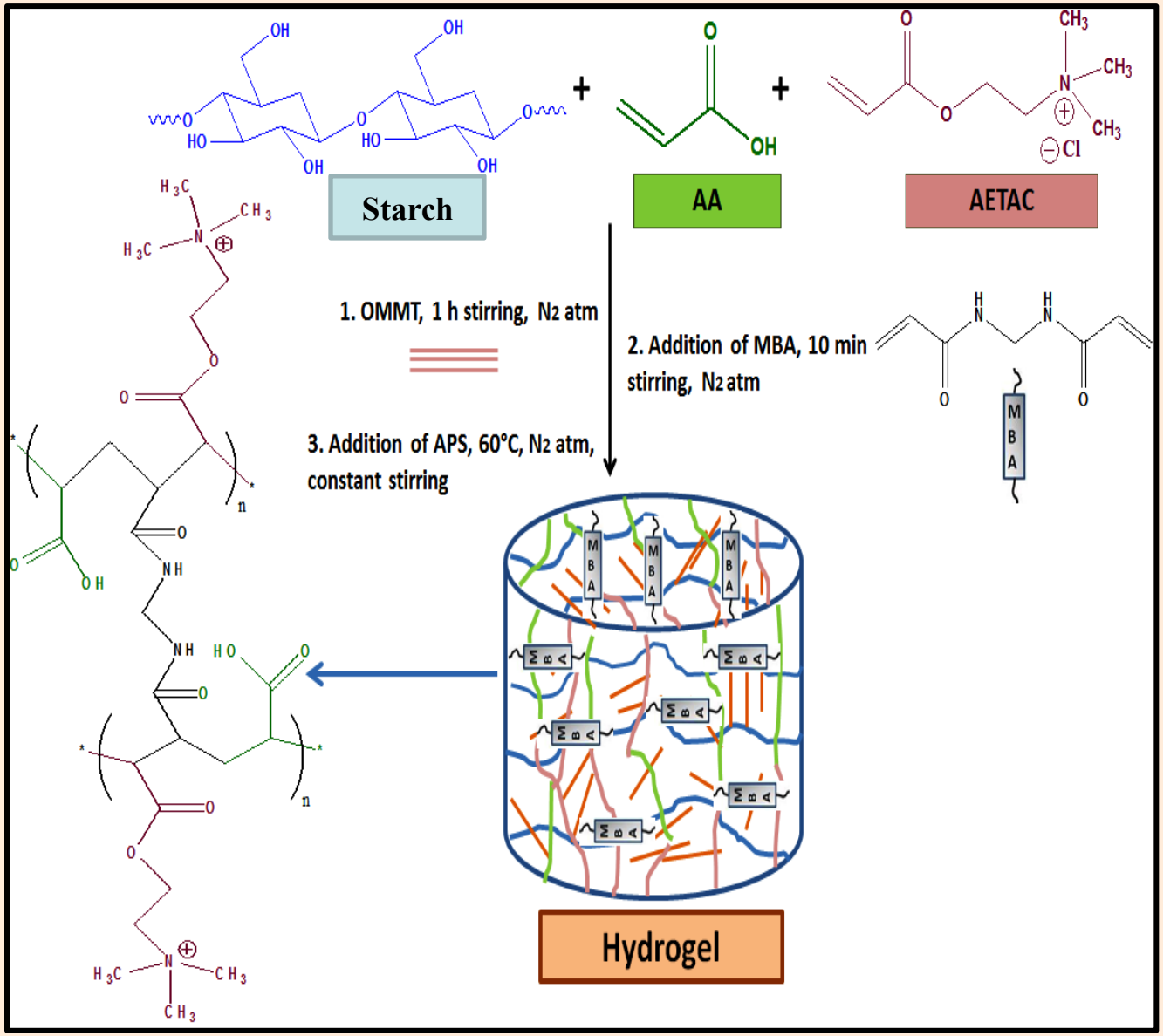


MBA



Prepared hydrogel

Scheme of hydrogel preparation



$$\text{Yield\%} = W_d / W_i \times 100$$

$$\text{Gel\%} = W_d / W_c \times 100$$

$$\text{Sol\%} = (100 - \text{Gel\%})$$

W_c = Constant weight of the hydrogel after drying;
W_i = the weight of total monomer taken;
W_d = Constant weight of the hydrogel after equilibrium swelling and subsequent drying

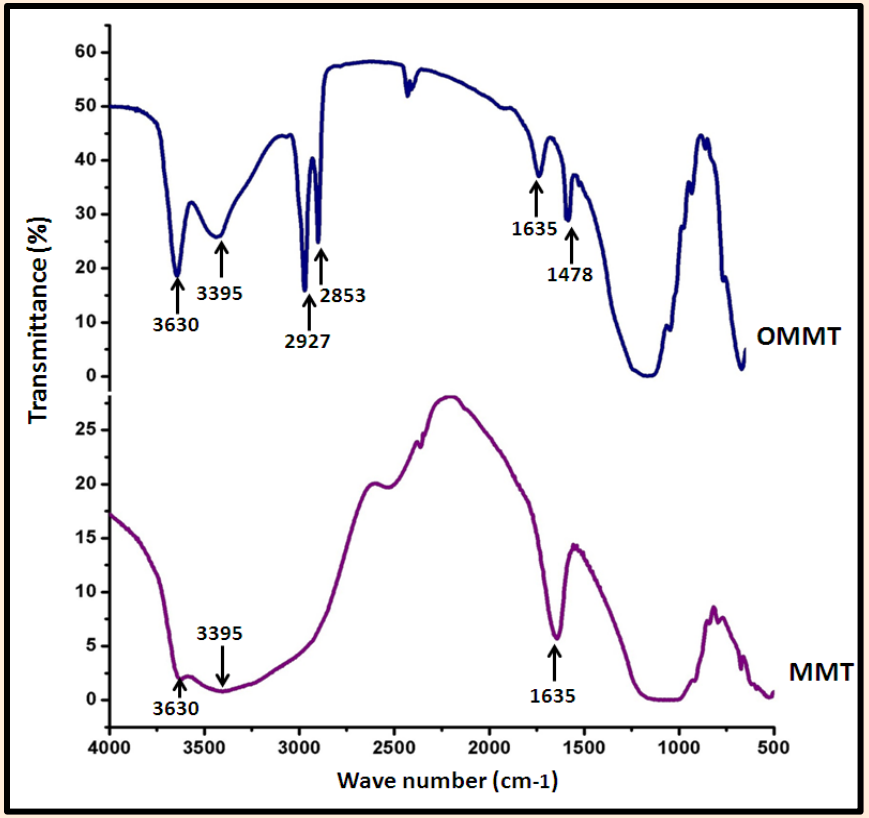
Yield % = 90
Gel % = 85

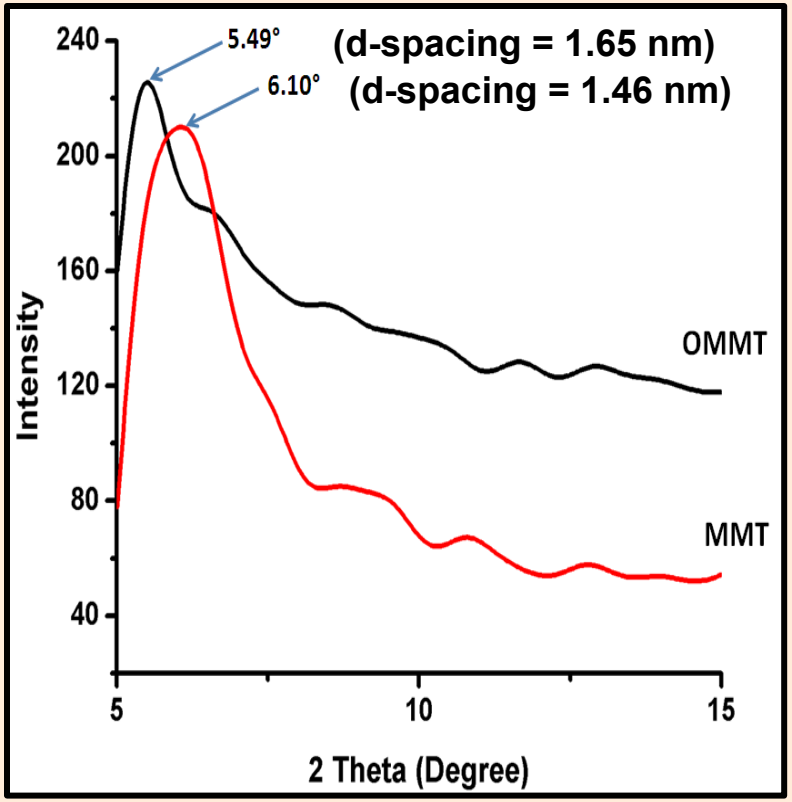
Composition of different hydrogel

Sample code	Starch (wt%)	AA : AETAC	MBA (wt% w.r.t total monomer)	OMMT (wt% w.r.t monomer)
S_{Base}	2.0	70:30	1.0	0.0
S_0	2.0	70:30	1.0	2.5
S_1	2.0	70:30	1.0	5.0
S_2	2.0	70:30	1.0	7.5
S_3	2.0	70:30	1.5	5.0
S_4	2.0	70:30	2.0	5.0
S_5	0.0	70:30	1.0	5.0
S_6	3.0	70:30	1.0	5.0
S_7	2.0	50:50	1.0	5.0
S_8	2.0	90:10	1.0	5.0

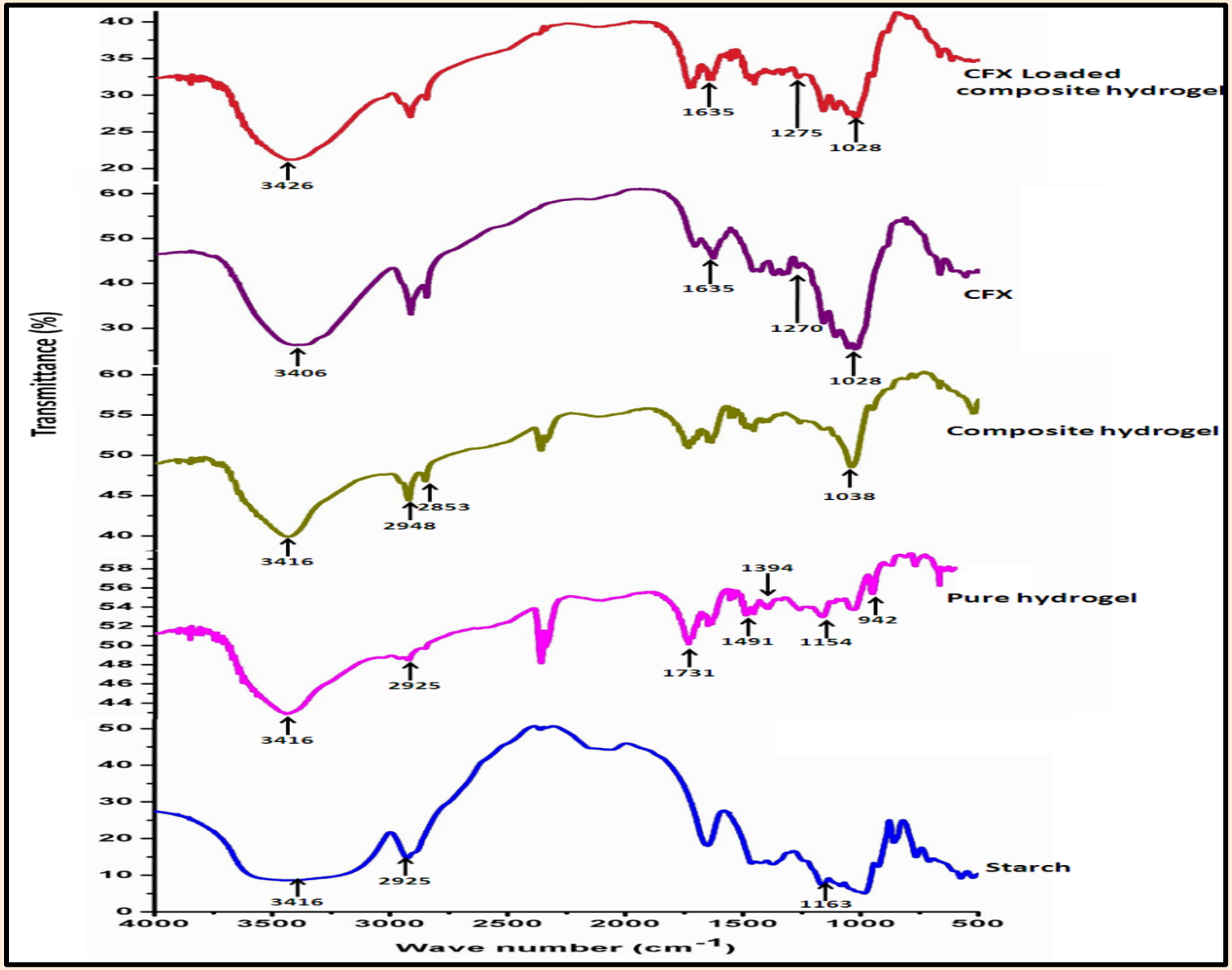
Total batch volume = 20 ml; APS = 1 wt% w.r.t monomer amount

Characterization of prepared OMMT (FTIR and XRD analysis)

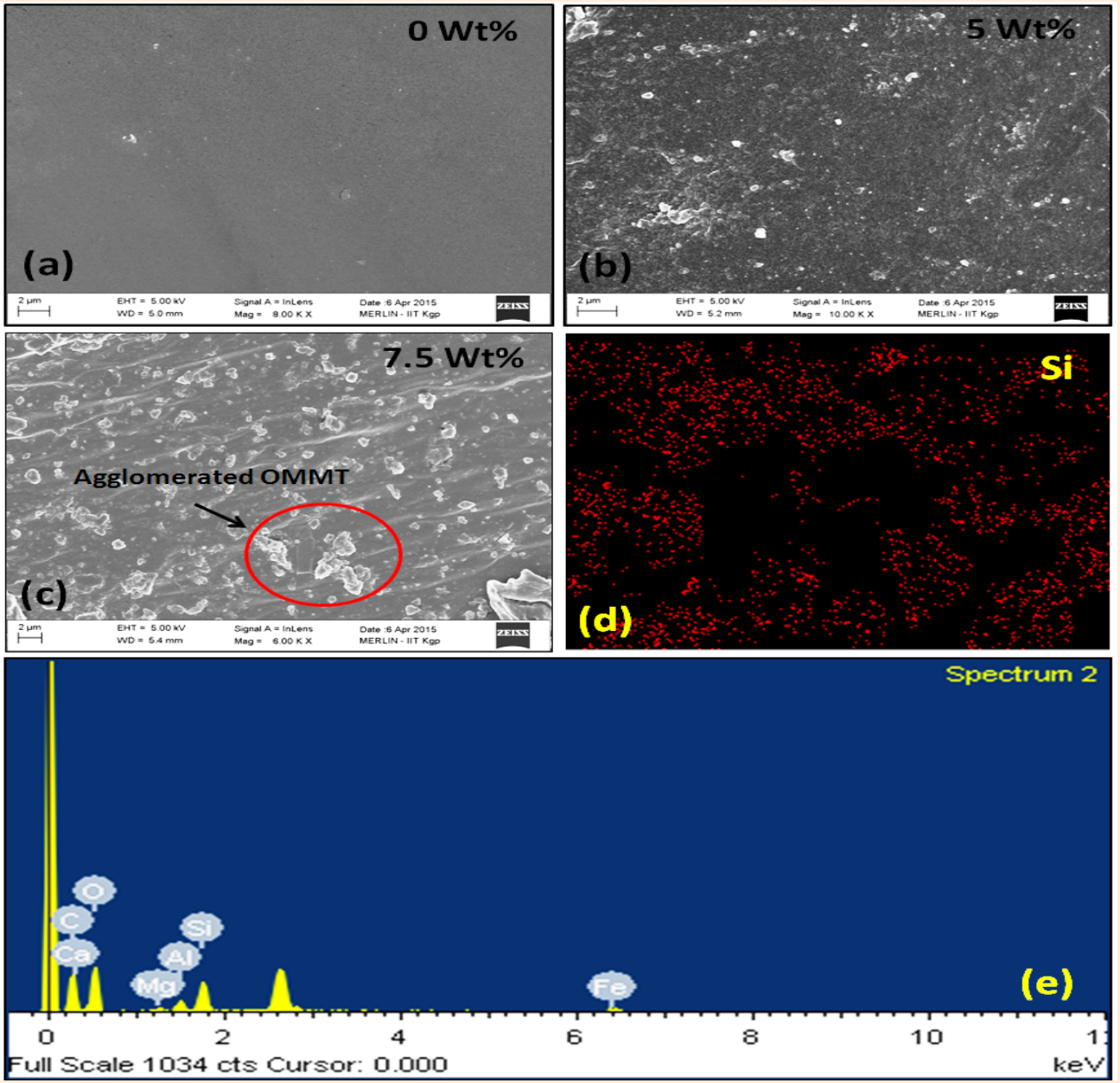




Characterization of prepared hydrogel (FTIR)



Characterization of prepared hydrogel (FESEM Analysis)



SEM analysis of

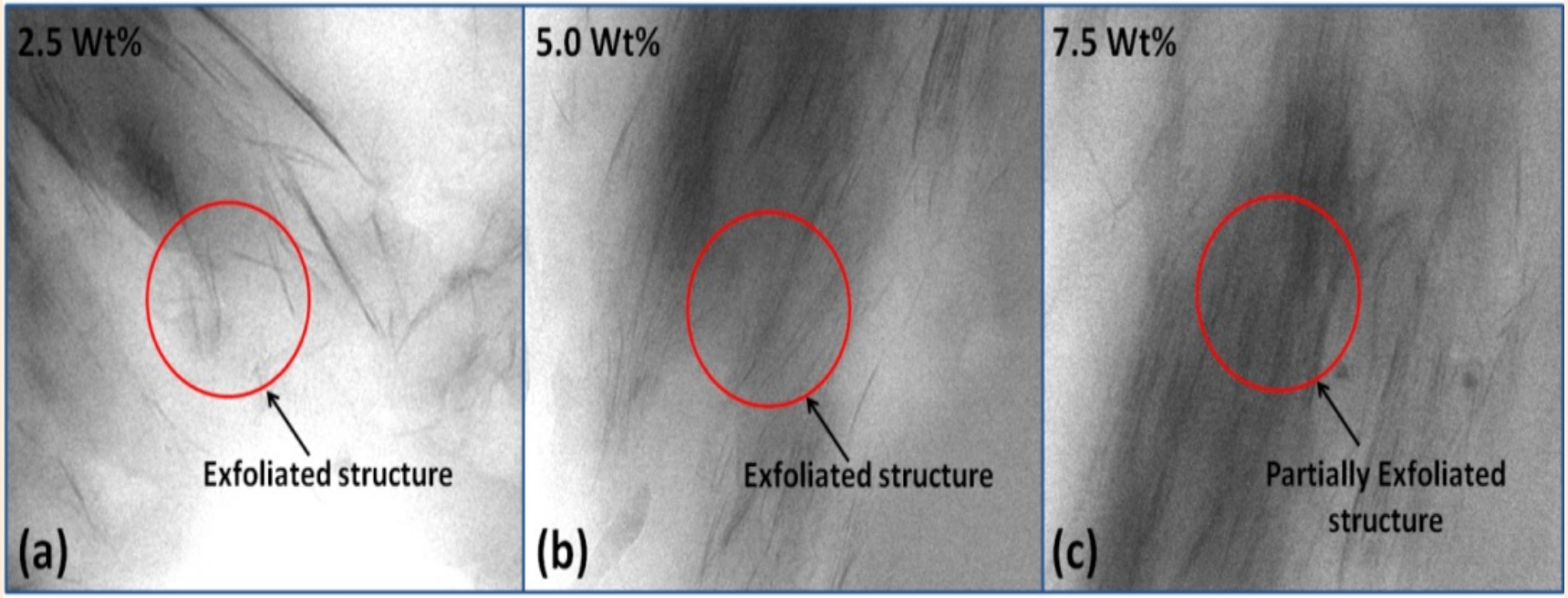
(a) Hydrogel without containing filler,

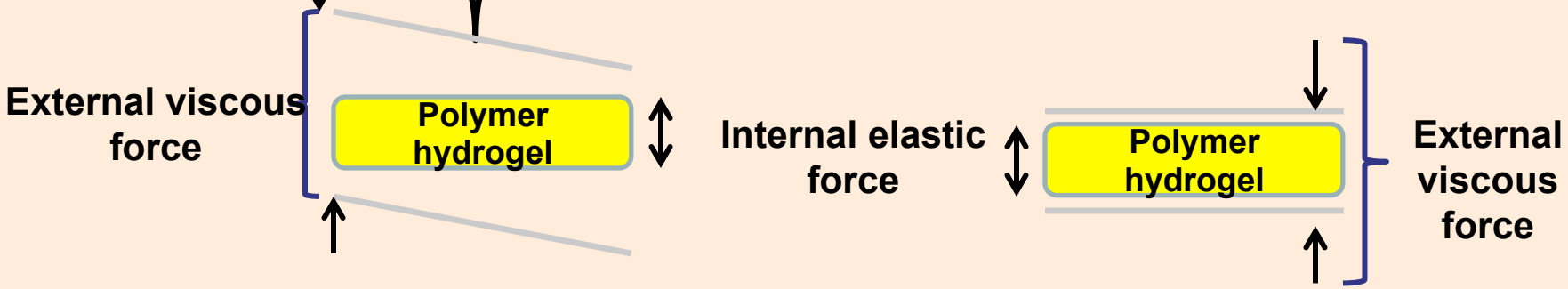
(b) & (c) Composite hydrogel with different content of OMMT,

(d) Bulk phase elemental mapping of the filler loaded hydrogel.

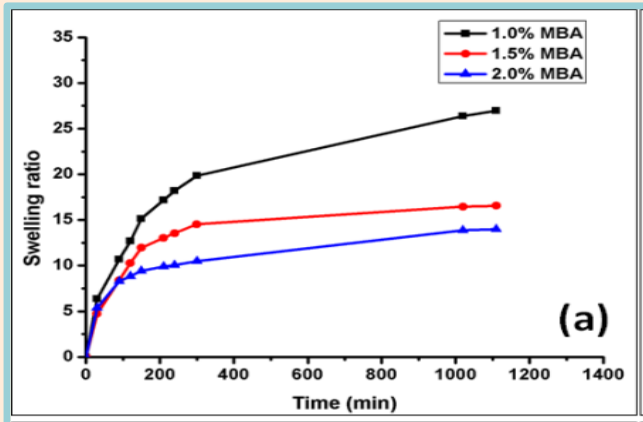
(e) EDAX analysis of filler loaded hydrogel.

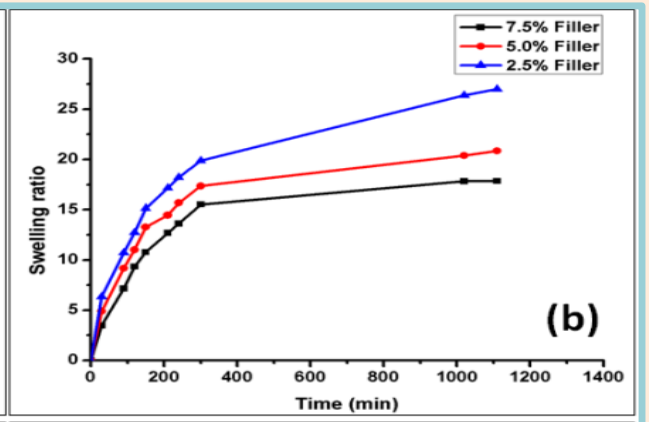
Characterization of prepared hydrogel (TEM Analysis)

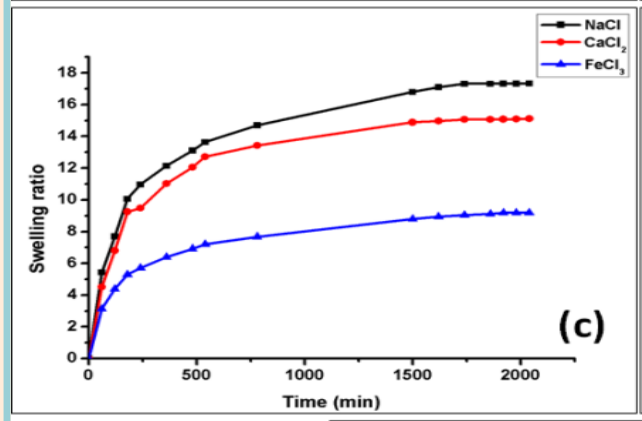


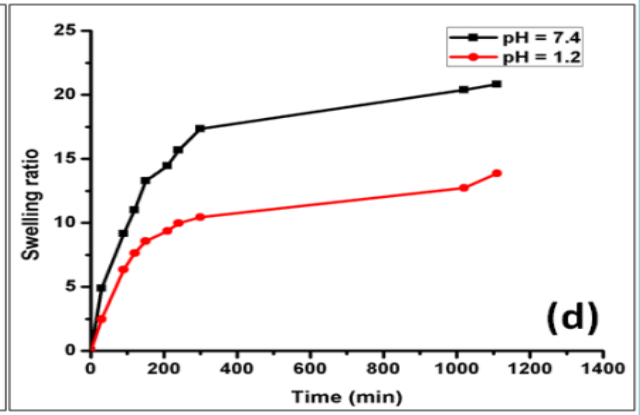


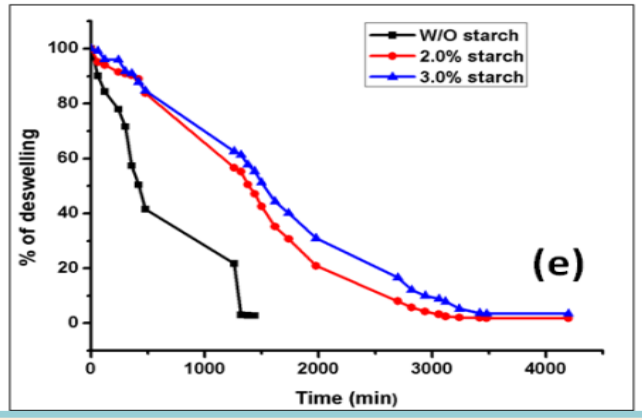
Swelling and deswelling study











Swelling study of the prepared hydrogel with variation in

a) **Different amount of crosslinker,**

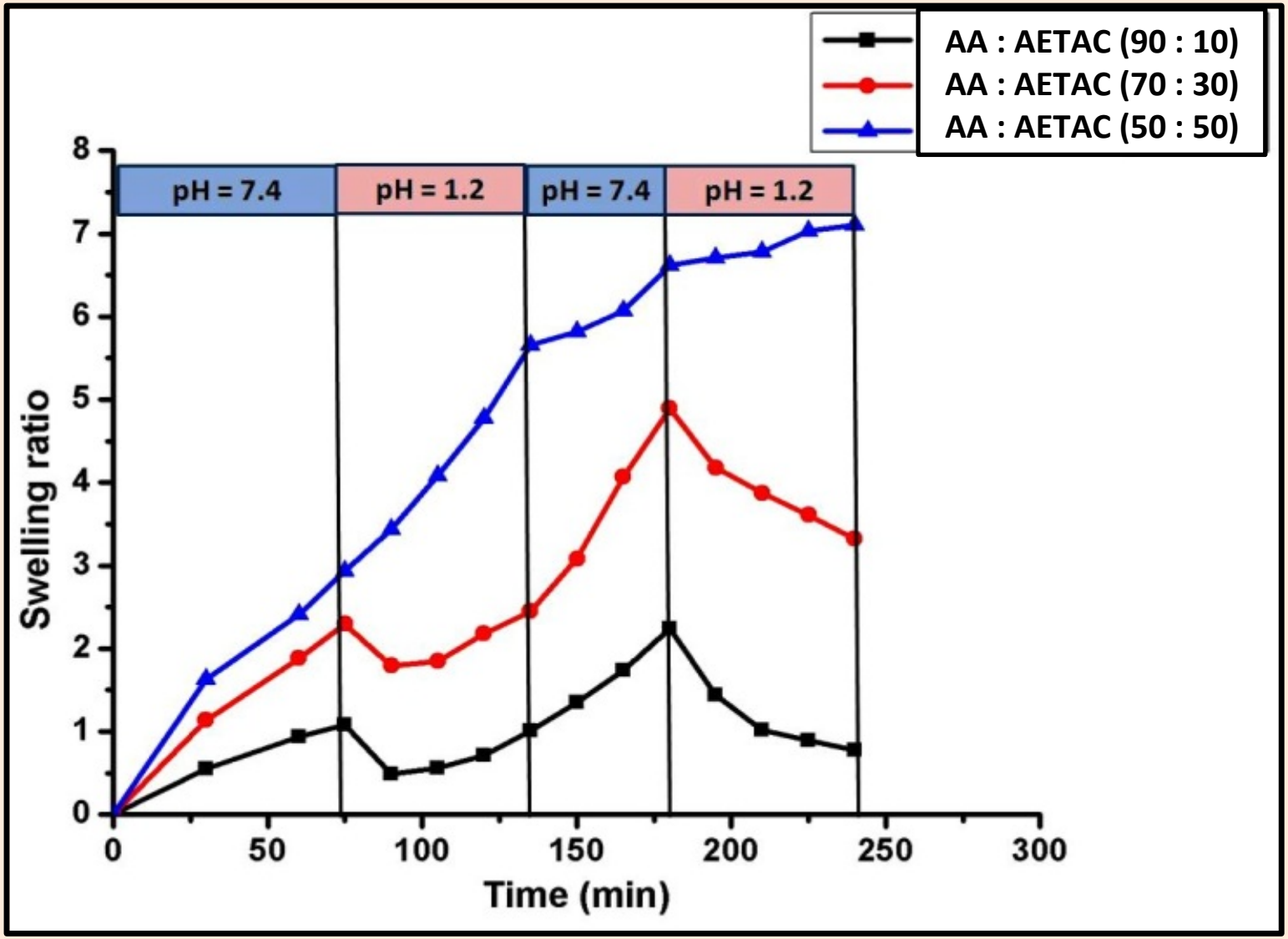
b) **in different amount of OMMT.**

c) **Swelling study of hydrogel in different salt solution.**

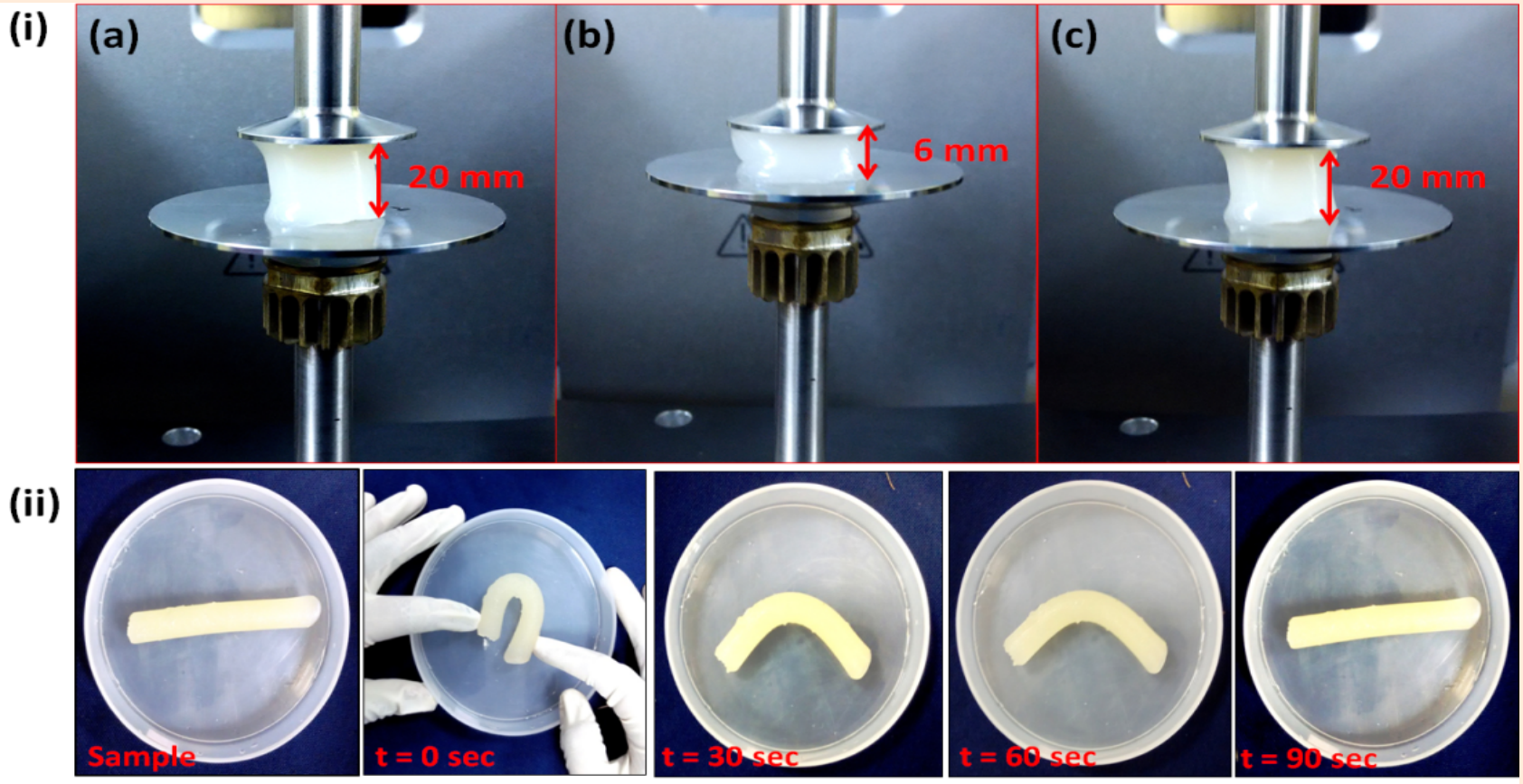
d) **Swelling study of the hydrogel containing 5 wt% OMMT at pH=1.2 and pH=7.4 solution.**

e) **Deswelling behavior of the hydrogel at varying content of starch.**

Study of pH responsiveness

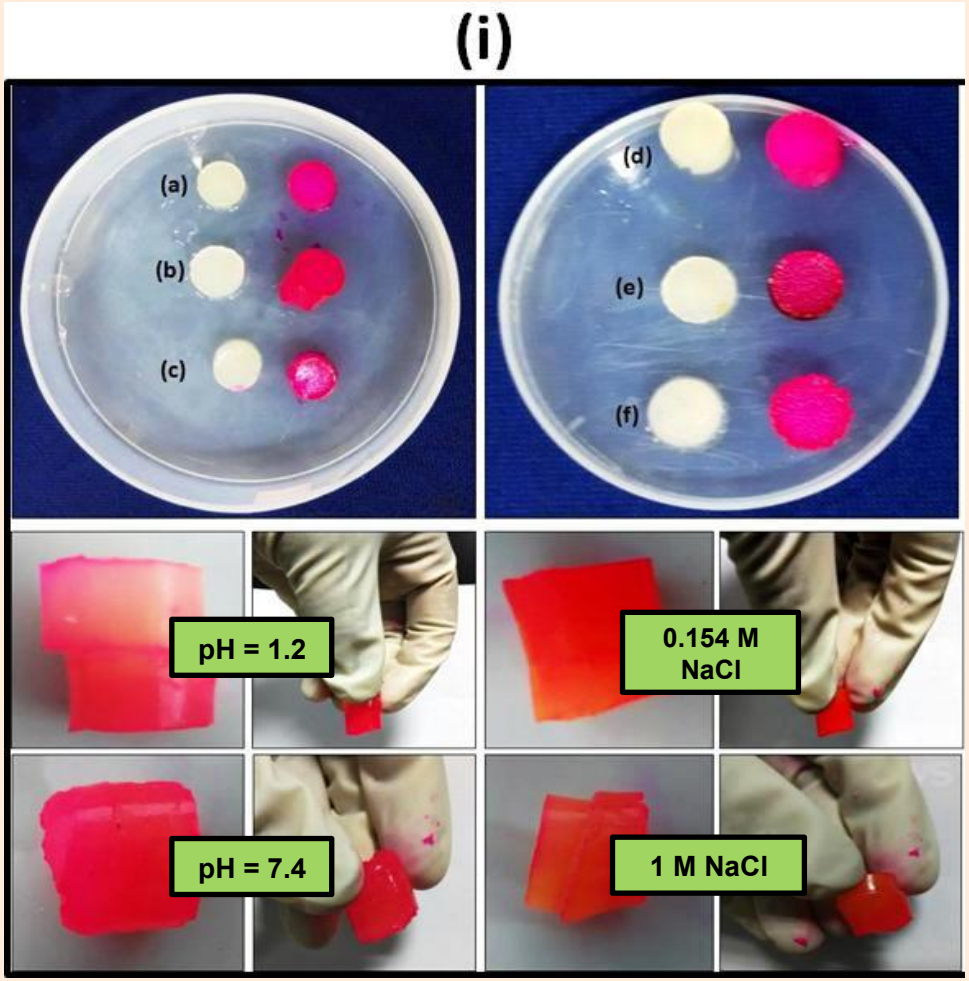


Compression set and bending study of the hydrogel

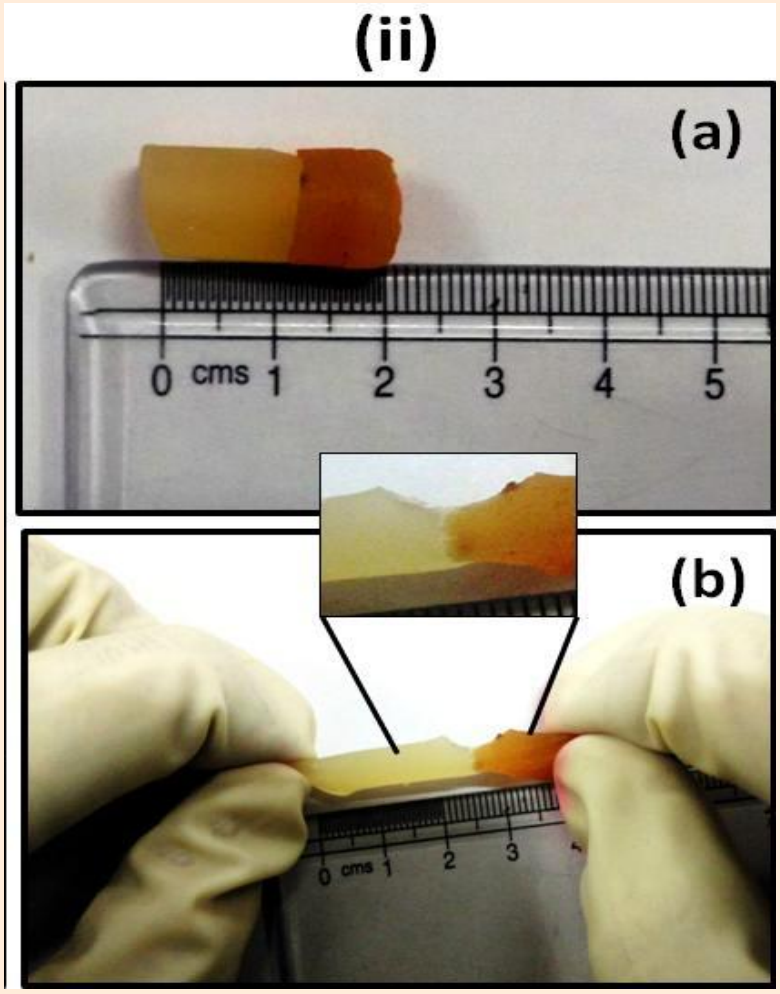


(i) Image of compression set study of the composite hydrogel a. before compression, b. during compression and c. after compression. (ii) Bending test of the composite hydrogel.

Self healing study of the hydrogel



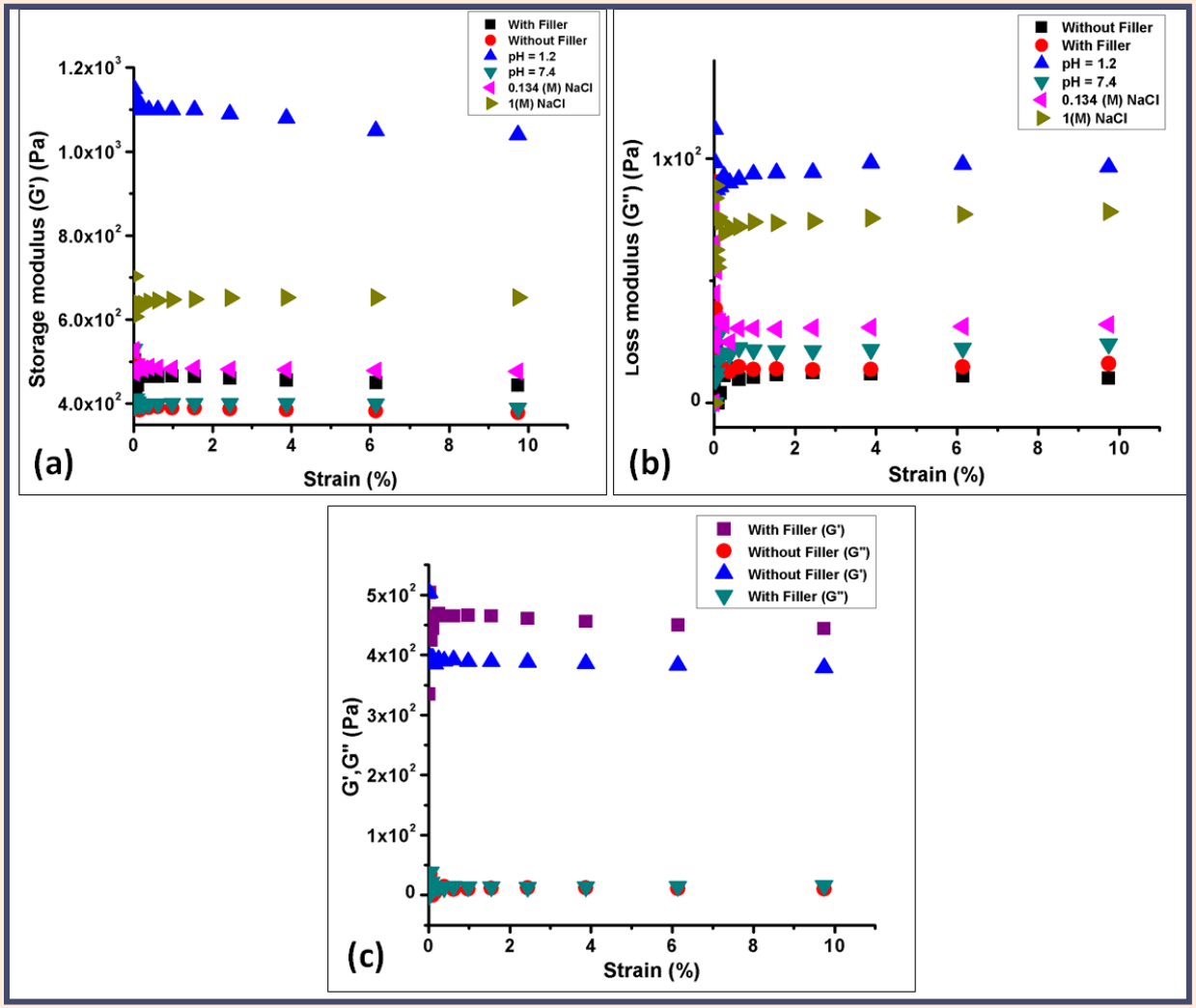
Self-healed hydrogel in different pH

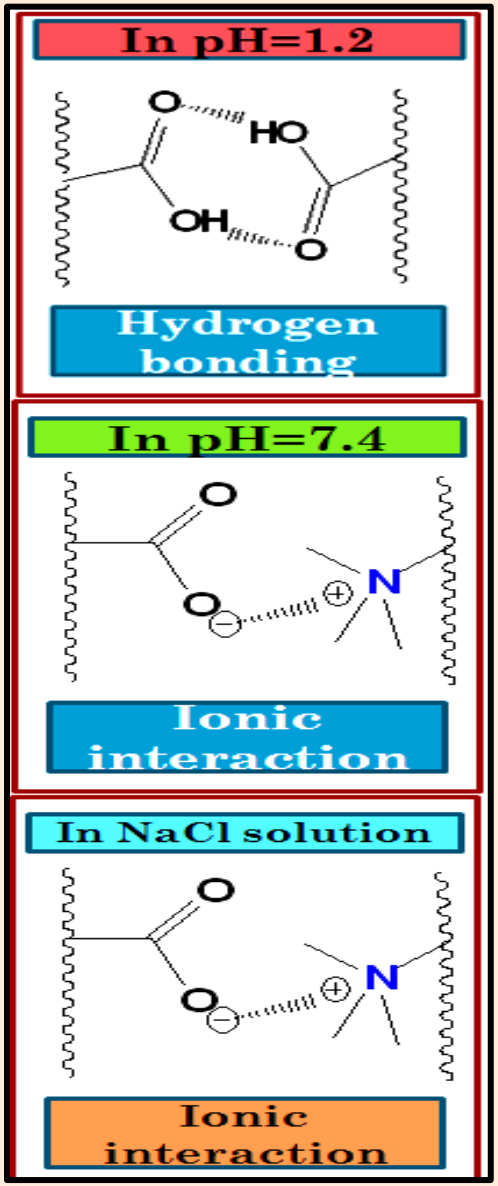


Elongation study of self-healed hydrogel

i) Self healing study of the hydrogel at different conditions a) pH 1.2 b) pH 7.4 c) 0.154 (M) NaCl soln. d) 1 (M) NaCl soln. e) Only Acrylic acid based gel f) Only AETAC based gel

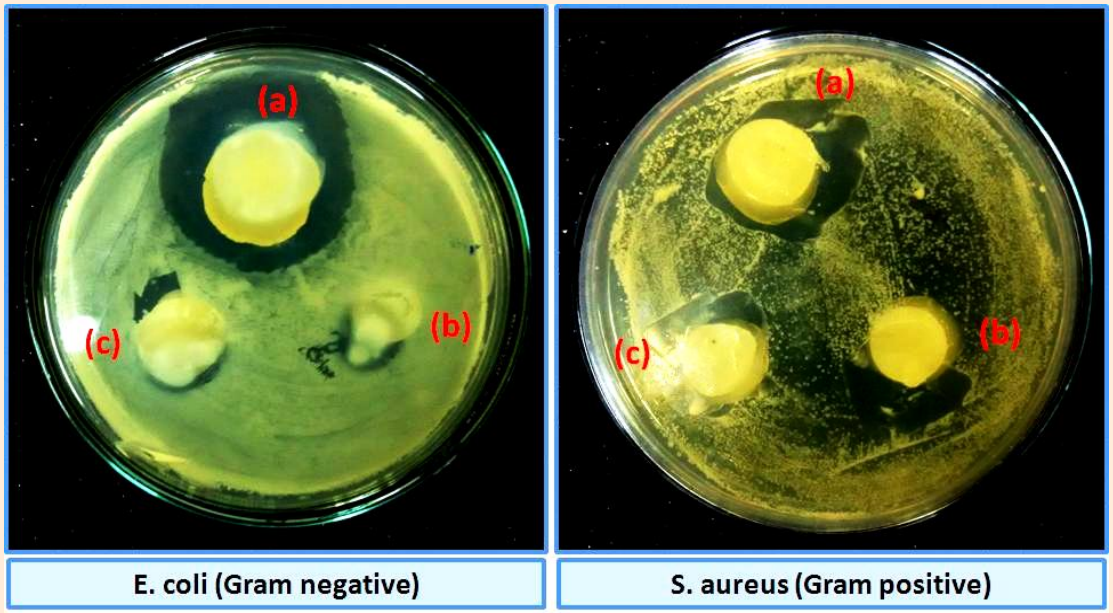
Rheological study of the hydrogel





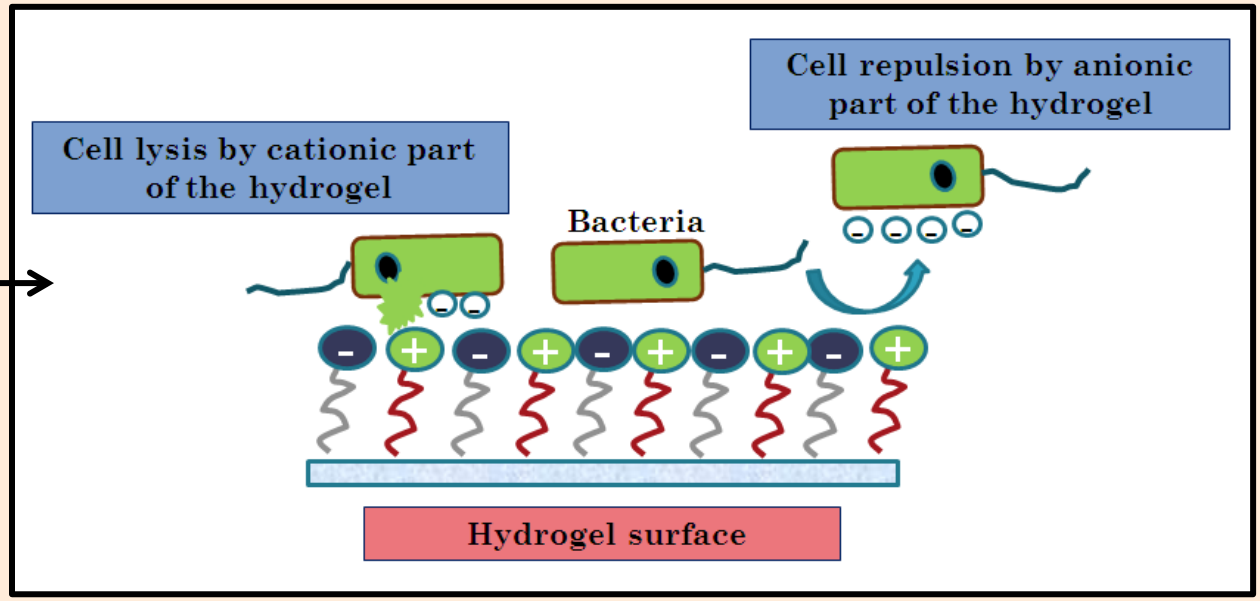
(a) Storage modulus (G') change vs Strain in uncut hydrogel and self-healed hydrogel, (b) Loss modulus (G'') change vs Strain in uncut hydrogel and self-healed hydrogel and (c) Plot of G' & G'' vs Strain for pure hydrogel and composite hydrogel containing 5.0 wt% filler.

Non-leaching Antimicrobial study

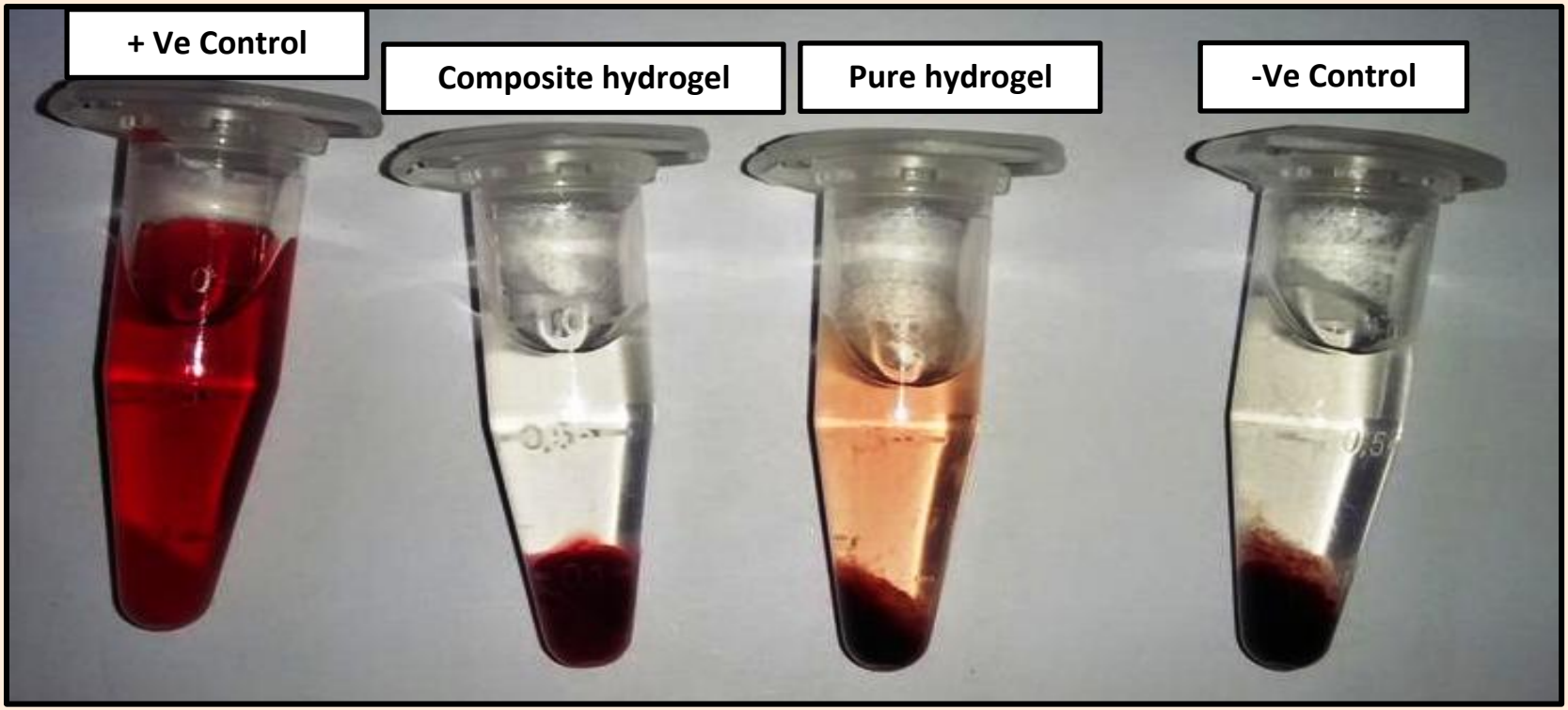


a) Ciprofloxacin loaded hydrogel
b) pure hydrogel
c) OMMT incorporated hydrogel

Mechanism of non-leaching antimicrobial activity



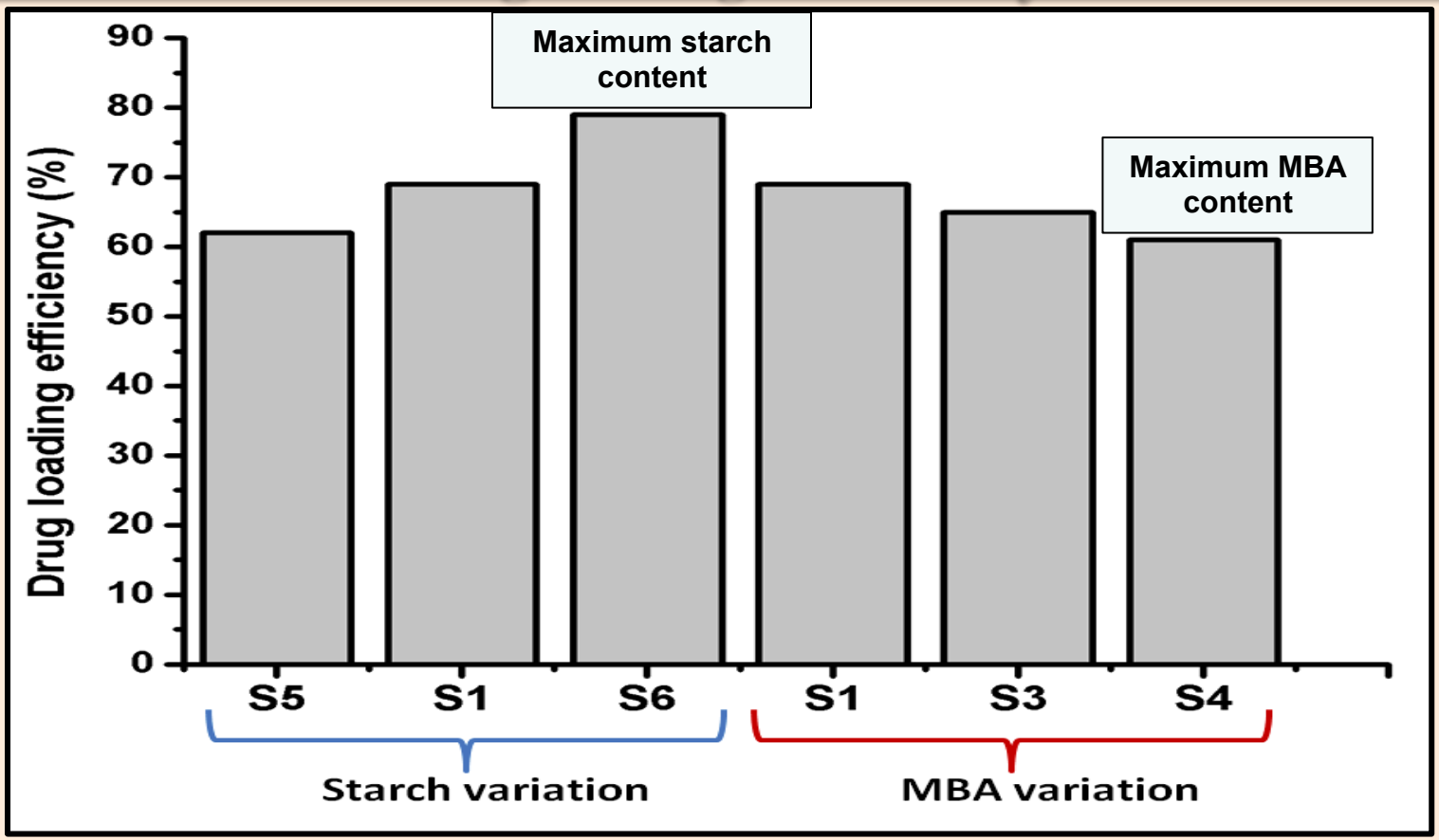
Haemocompatibility study



Hemolysis ratio (%) = *Suspensions↓Abs* –Negative *control↓Abs* /Positive *control↓Abs* –Negative *control↓Abs* X 100

- **For the composite hydrogel hemolysis ratio = 5%**
- **For the pure hydrogel hemolysis ratio = 12%**

Drug loading efficiency



Drug loading (DL) % (mg per gm of the hydrogel) = $W_d - W_i / W_i \times 100$

W_d = Weight of fully swelled hydrogel in drug solution (1 mg/ml).

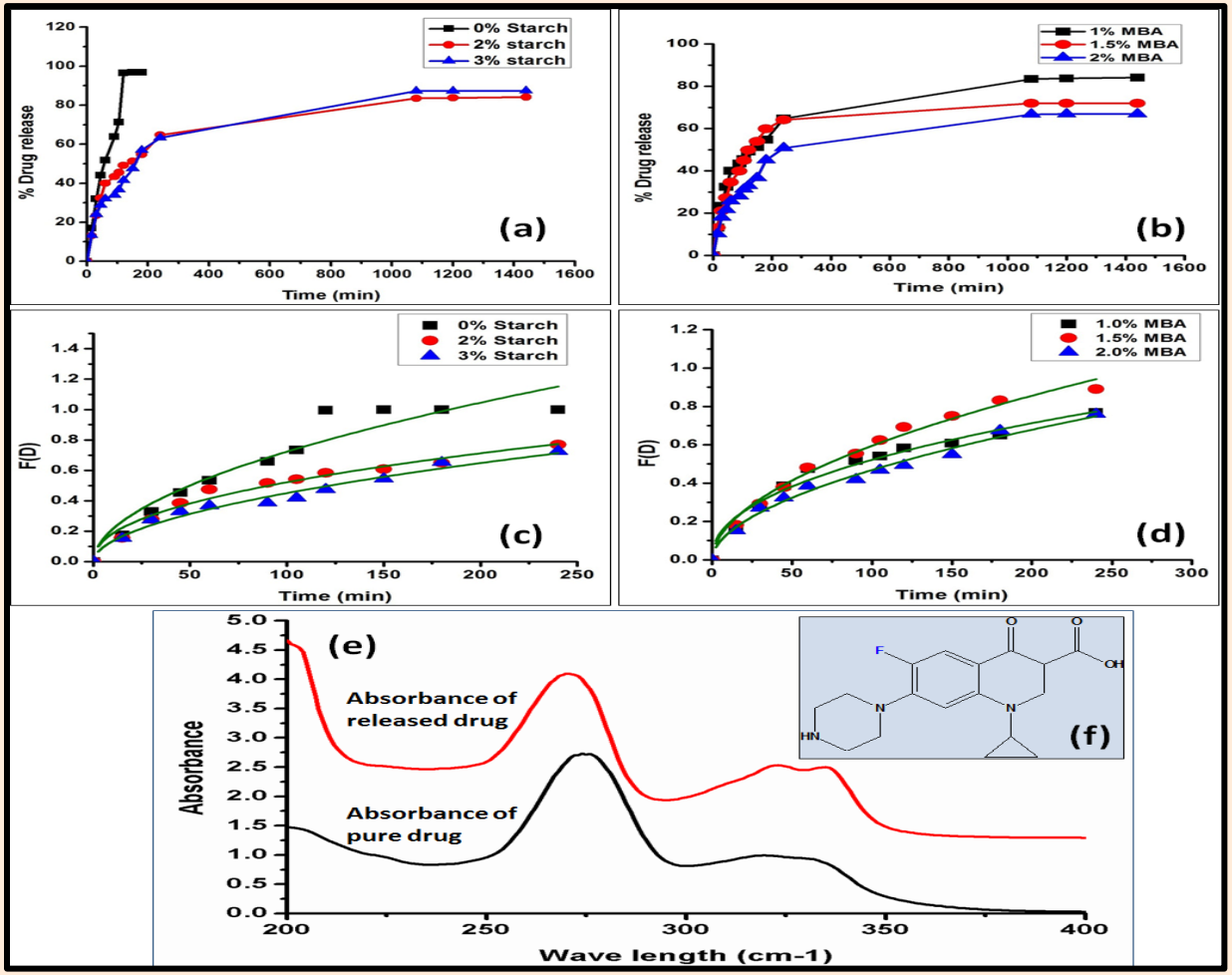
W_i = Weight of dry hydrogel.

Drug release study

Drug release% =
$W_{drug} - W_{release} / W_{release} \times 100$

$F(D) = M_{Dt} / M_{De} = K_{KP} t^{n}$

M_{Dt} = Amount of drug release at t time;
M_{De} = Amount od drug release at equilibrium condition;
"n" = Diffusion exponent;
In our case "n" in avg. = 0.456

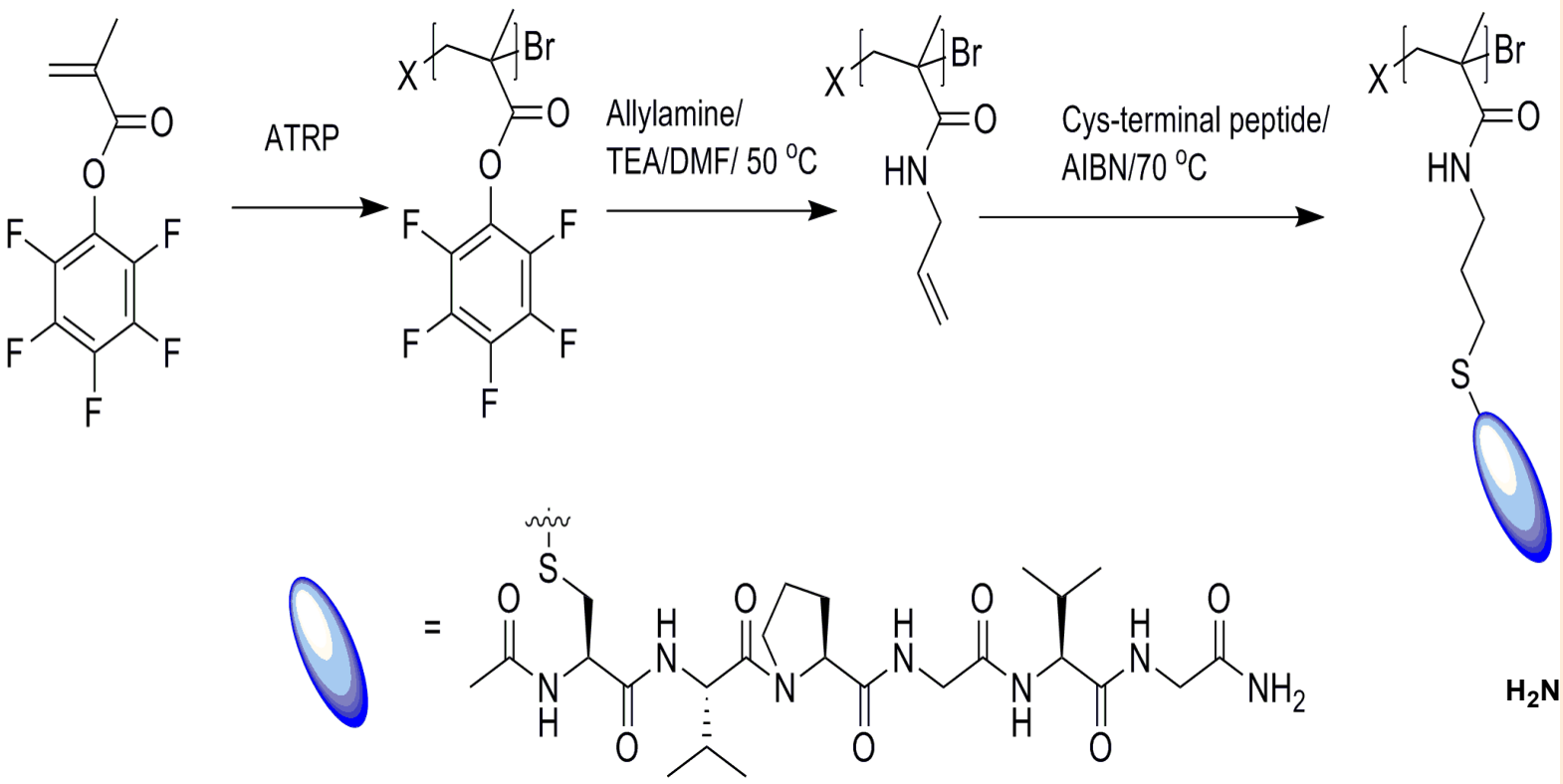


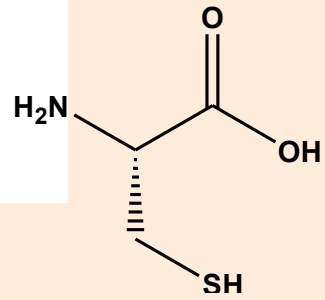
Drug release study at (a) different starch loading, (b) different crosslinker loading and respective (c & d) Korsmeyer–Peppas model fitting. (e) UV-vis spectra of pure and released drug. (f) Chemical structure of ciprofloxacin drug.

Polymer-Peptide Conjugate Material via CRP & Thiol-ene Reaction

- Polymer-Peptide hybrid materials are useful
  - Drug delivery
  - Substrates for Cell Adhesion & Recognition of certain proteins
  - Medical applications (treatment of different diseases) .
- Elastin is a protein available in mammalian tissues, like in skin, lungs, ligaments. Its primary structure has repeating pentapeptide, VPGVG (V = valine, P = proline, G = glycine)
- Polymers with Elastin based side chain have important applications, like smart materials etc.

Peptide-Polymer Conjugate via Tandem "Ester-Amide / Thiol-Ene" Post-Polymerization Modification of PPFMA



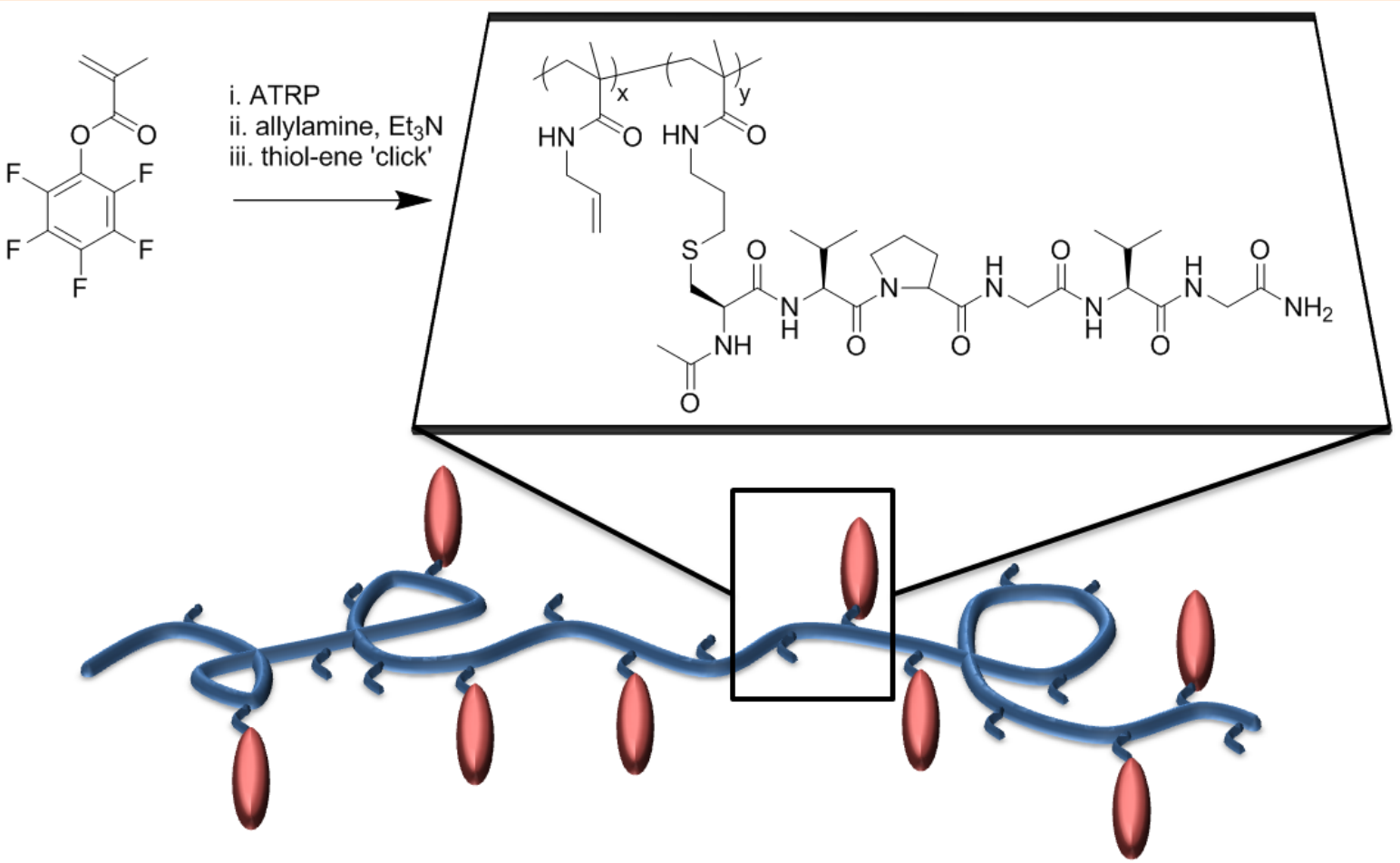


Cysteine

Peptide = -CVPGVG (C = Cysteine, V = Valine, P = Proline; G = Glycine

Biomacromolecules 2011, *12*, 2908

Polymer-peptide conjugate via Tandem Post-polymerization Modification



Biomacromolecules 2011, *12*, 2908

Conclusions

- From the swelling study it was observed that swelling ratio of the hydrogel in acidic medium is lower than swelling in pH = 7.4 which is attributed due to presence of H-bonding in acidic condition between the carboxylic groups which increase the cross linking density of the gel.

- From the antimicrobial study it can be observed that presence of quaternary ammonium ion group in the hydrogel effectively destroy both the gram positive and gram negative bacteria.

- From blood compatibility study it is observed that, synthesized hydrogel is very much blood compatible.

- From drug release study it is observed that presence of starch sustained the release of drug from the bulk of hydrogel.

- Successful "Click Chemistry" of Diels Alder reaction was carried out to demonstrate thermoreversible self-healing properties.

- ATRP & Thiol-ene Reaction were successfully used to prepare Peptide-Polymer Conjugate.

Acknowledgement

DST, New Delhi
CSIR, New Delhi
BARC, Mumbai
VSSC, Trivandrum
DMSRDE. Kanpur
Asian Paints, Mumbai

USA-India Education Foundation (USIEF)
DSM Elastomers, The Netherlands
Dutch Polymer Institute,
Eindhoven University of Technology
TNO Industries, Netherlands
Royal Society, UK,
DFG & DAAD, Germany
EPFL, Switzerland

Prof. S. Rimmer, Bradford, UK
Prof. Brigitte Voit, IPF, Dresden, Germany
Prof. H-A. Klok, EPFL, Switzerland
Prof. Jimmy Mays, UTK, USA

Acknowledgement

Mr. Sovan Banerjee, Mr. Souvik Ata, Dr. (Ms.) A. Kavitha, Mr. Nabendu Pramanik, Mr. Arindam Chakraborty

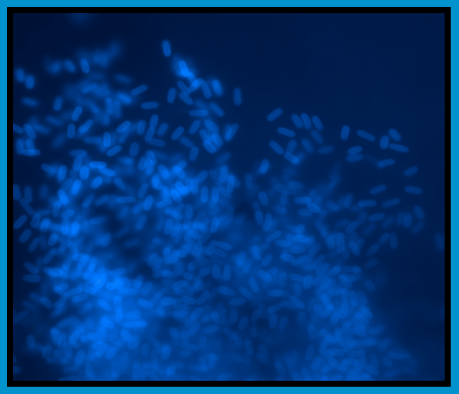
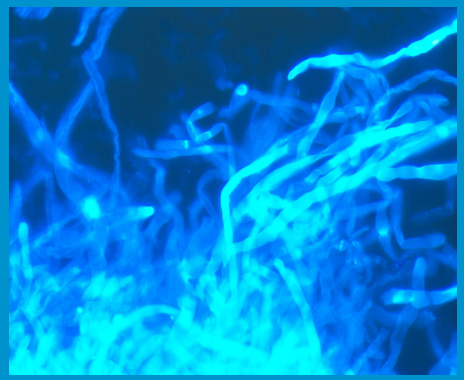
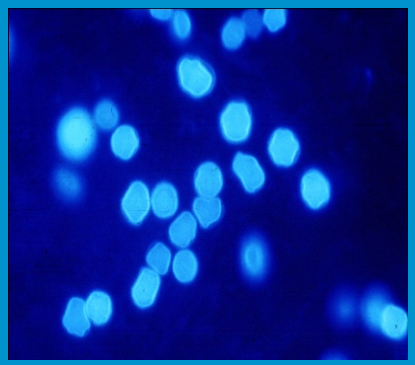
- **Dr. (Ms.) Haimanti Dutta, Dr. Sambhu Bhadra, Dr.(Ms.) A. Kavitha, Dr. M. Thirumal, Dr. Rajesh Babu, Dr. Sanjay Dutta, Dr. Anjan Biswas, Dr. Dhruba J. Haloi, Dr. Ms. Sangita Singh, Dr. Subhendu Bhandari, Mr. Bishnu Koiry, Dr. Prithwiraj Mandal, Mr. Souvik Ata, Mr. Nabendu Pramanik, Mr. Prasanta Behera, Mr. Sovan Banerjee Mr. P. Siva, Mr. Girish Mirchandani,**

- **Mr. Satya Sadhan Dutta**
- **Master Students (20)**


Thank you

LVPEI
So that all may see

Ocular Pathogens: Challenges beyond bacteria

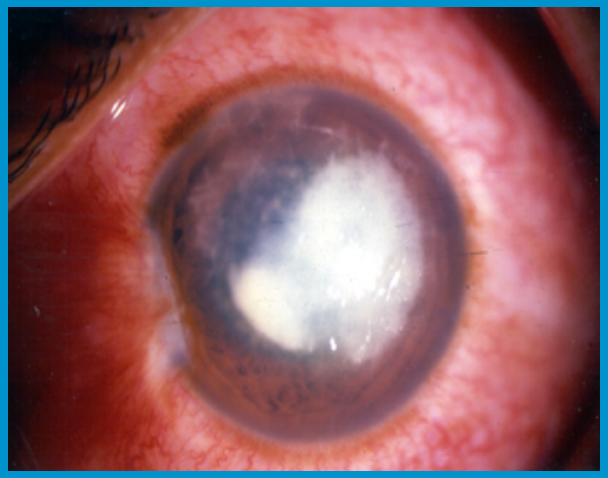




Savitri Sharma, MD, FAMS
L V Prasad Eye Institute
Hyderabad
India

- Corneal blindness accounts for 15.4% of blindness in India (Lancet1998)
- Incidence of corneal ulceration is 11.3/10,000 population in south India (Whitcher et al. Br J Ophthalmol, 1997)
- Microbial keratitis:
  - Bacterial
  - Fungal
  - Parasitic
  - Viral

Relevant to this meeting

- Fungal/Mycotic Keratitis:
  - Treated with antifungal antibiotics
  - Poor response to medical treatment
  - Antifungal resistance not well studied
  - Effective drugs and drug delivery systems required
- Parasitic infections that need more effective drugs



Reported Prevalence of Keratitis

	India[1] (434)	Nepal[2] (405)	Ghana[3] (290)	USA[4] (663)	Australia[5] (291)	LVPEI[6] (5,897)
Culture +ve	297	324	146	371	112	3,563
Bacteria (%)	47.1	79.0	24.7	64.2	90.2	51.9
Fungi (%)	46.8	8.4	71.9	35.8	6.2	38.2
Mixed (%)	5.1	12.6	2.7	---	---	7.5
Acanthamoeba (%)	1.0	---	0.7	---	3.6	2.4
Microsporidia(%)						0.4

1. Srinivasan M, *et al.* Br J Ophthalmol 1997; 81: 965-971.
2. Upadhyay MP, *et al.* Am J Ophthalmol 1991; 111: 92-99.
3. Leck AK, *et al.* Br J Ophthalmol 2002; 86: 1211-1215.
4. Liesegang TJ, *et al.* Am J Ophthalmol 1980; 90: 38-47.
5. Keay L, *et al.* Ophthalmology 2006; 113: 109-116.
6. Gopinathan U, *et al.* Indian J Ophthalmol 2009; 57: 273-279.

Distribution of species of fungi in Fungal Keratitis

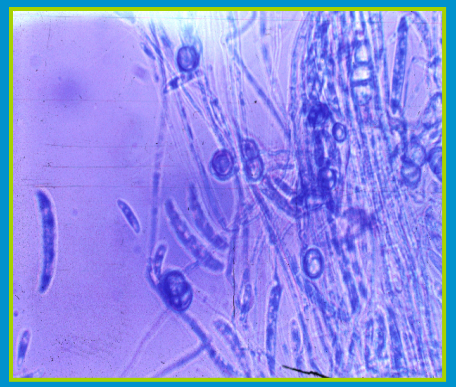
Tropical areas - filamentous fungi
Temperate zones - Yeast

Organisms	India[1]	Nepal[2]	Ghana[3]	USA[4]	Australia[5]	LVPEI[6]
Moulds (%)	100	86.8	98.5	33	61	99.2
Aspergillus (%)	16.1	47.0	15.3	12	17.1	28.9
Fusarium (%)	47.1	11.7	52.3	---	14.3	35.6
Yeast (%)	---	13.2	1.5	67	39	0.8

1. Srinivasan M, *et al.* Br J Ophthalmol 1997; 81: 965-971.
2. Upadhyay MP, *et al.* Am J Ophthalmol 1991; 111: 92-99.
3. Leck AK, *et al.* Br J Ophthalmol 2002; 86: 1211-1215.
4. Ritterband DC, *et al.* Cornea 2006; 25: 264-267.
5. Bhartiya P, *et al.* Clin Experiment Ophthalmol 2007; 35: 124-130.
6. Gopinathan U, *et al.* Indian J Ophthalmol 2009; 57: 273-279.

Mycotic Keratitis, n=1352

Fungal species	No.	(%)
Hyaline	1133	(83.3)
Fusarium spp.	506	(37.2)
Aspergillus spp.	417	(30.7)
Acremonium spp.	12	(0.9)
Chrysosporium spp.	5	(0.4)
Rhizopus spp.	1	(0.1)
Unidentified hyaline	192	(14.1)

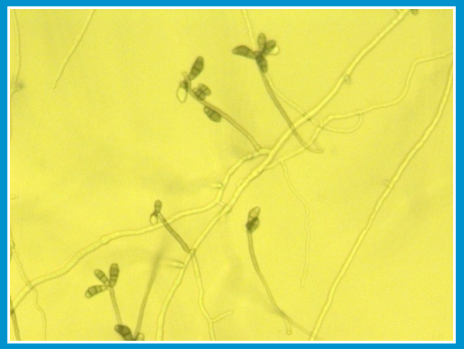


Gopinathan U, et al. Indian J Ophthalmol 2009; 57(4): 273-279

Mycotic Keratitis, n=1352

Fungal species	No	(%)
Dematiaceous	218	(16)
Curvularia spp.	39	(2.8)
Bipolaris spp.	15	(1.1)
Exserohilum spp.	1	(0.8)
Lasiodiplodia spp.	7	(0.5)
Cladosporium spp.	6	(0.4)
Scedosporium spp.	5	(0.4)
Alternaria spp.	4	(0.3)
Unidentified dematiaceous	124	(9.5)
Yeast	9	(0.7)

Bipolaris spicifera

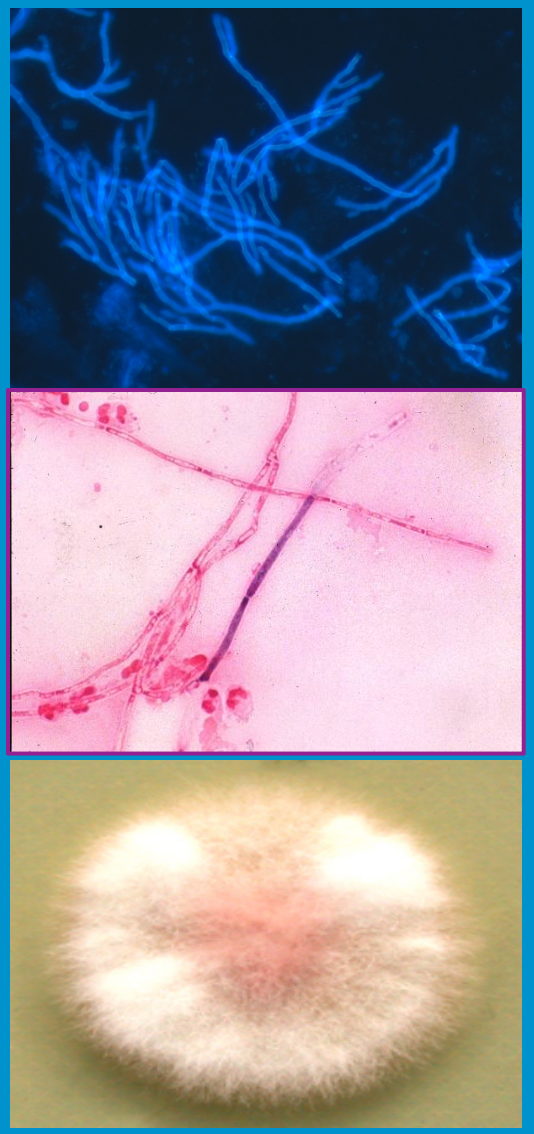


Curvularia lunata

Gopinathan U, Sharma S, et al. Indian J Ophthalmol 2009; 57(4): 273-279

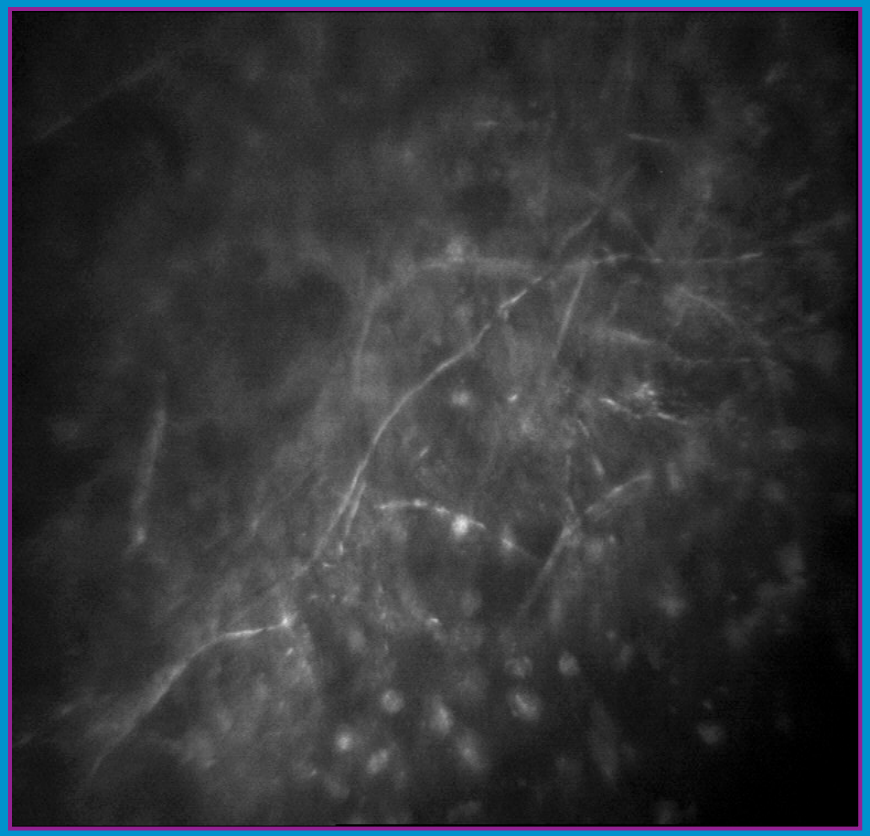
Laboratory Diagnosis of Fungal Keratitis

- Simple laboratory tests such as microscopic examination of corneal scrapings and culture are highly sensitive (>90%) for diagnosis.
  - Calcofluor white stain
  - Gram stain
- Culture on blood agar, Sabouraud dextrose agar.



Confocal Microscopy

(In vivo diagnosis)



Vadavalli et al. Ophthalmology2011;118:29

Medical Therapy - Antifungal Drugs

Topical

- Polyenes – Amphotericin B (0.15%), Natamycin (5%)
- Azoles – Fluconazole, Itraconazole, Voriconazole, econazole (2%)
- Flucytosine

Systemic - Azoles, Amphotericin B, Echinocandins

Combination therapy of Azoles and Polyenes

Antifungal Drugs

Limitations

- Fungistatic nature
- Narrow spectrum
- Poor penetration
- Toxicity
- No conclusive advantage of one over another-Contradictory results
- Limited information on antifungal susceptibility of ocular isolates

In vitro Antifungal Susceptibility

- * 43 *Fusarium* spp: MIC 90

Ampho B	- 2 µg/ml
Natamycin	- 4
Miconazole	- >32
Itraconazole	- >32
Flucytosine	- >512

- @ Natamycin eye drops usage for MIC

* Reuben et al. antimicrob agents chemother1989;33:1647
@ Lalitha et al. J clin Microbiol 2008;46:3477
@ Pradhan et al. Indian J Ophthalmol 2011(In Press)

CLSI guidelines not available for many antifungal antibiotics

Antifungal drugs for fungal keratitis - RCT

(Inconsistent with *in vitro* results)

- * 112 patients- 2% econazole Vs 5% natamycin
- No difference in outcome. Equally effective
- @ 120 patients - 1% voriconazole Vs 5% natamycin
- No difference in outcome at 3 months.
- Voriconazole:
  - Excellent penetration in deeper layers of cornea
  - Soluble drug- anecdotal reports of intracorneal injection with good outcome.

* Prajna et al. Br J Ophthalmol 2003;87::1235
@ Prajna et al. Arch Ophthalmol 2010;128:672

Fungal Keratitis Management

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So that all may see

Cochrane review

- 12 randomised trials
- 981 subjects randomized
- Eight antifungal drugs studied
- All trials done in developing nations
- Variable quality and underpowered
- No good evidence for most comparisons except Natamycin and Voriconazole

Fungal Keratitis

LVPEI
So that all may see

Natamycin v/s Voriconazole

Outcome	Arora et al 2011		MUTT 2013		Prajna et al 2010	
Clinical cure	Nata N=15	Vori N=15	Nata N=162	Vori N=161	Nata N=60	Vori N=60
Number (%)	15 (100)	14 (93.3)	NR	NR	NR	NR
Microbiology cure	Nata N=15	Vori N=15	Nata N=155	Vori N=143	Nata N=60	Vori N=60
Number (%)	NA	NA	132 (85.2)	75 (52.1)	NA	NA

Fungal Keratitis Management

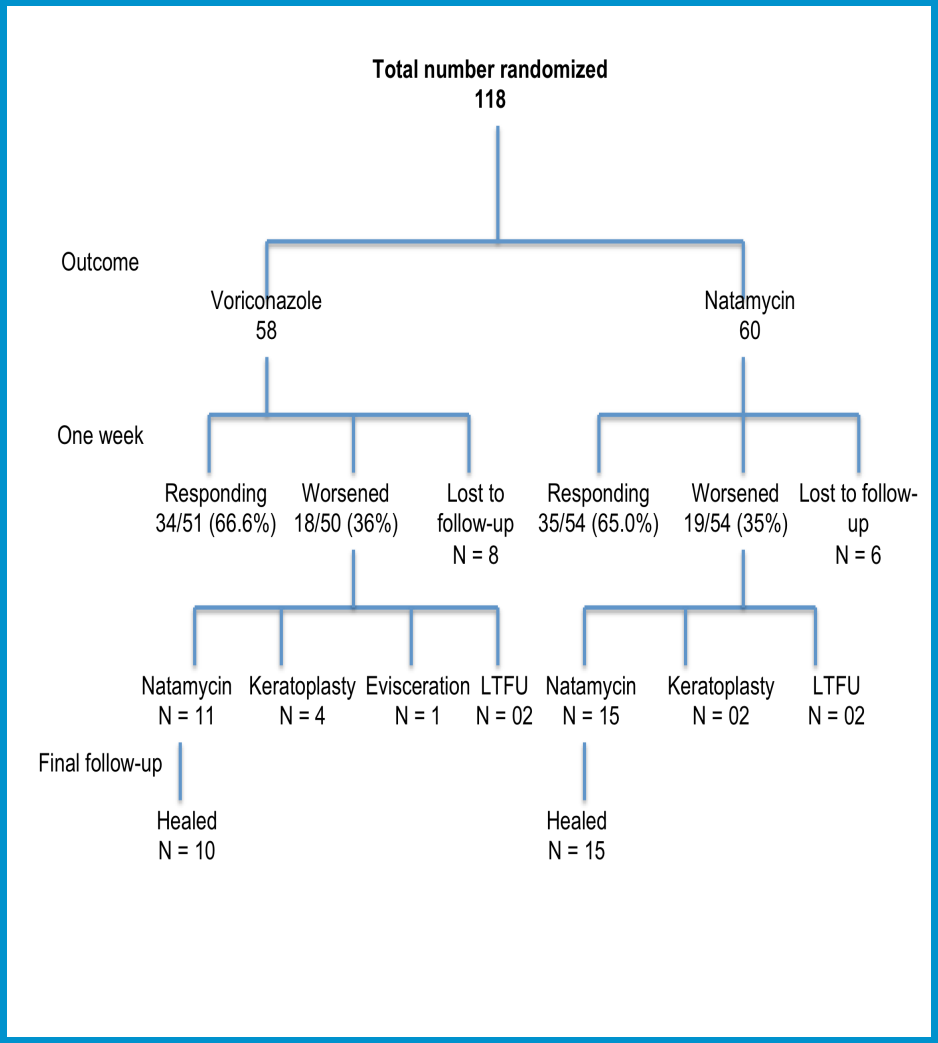
LVPEI
So that all may see

Re-appraisal of topical 1% voriconazole and 5% natamycin in the treatment of fungal keratitis in a randomised trial

Savitri Sharma,[1,2] Sujata Das,[1] Ajoy Virdi,[1] Merle Fernandes,[3] Srikant K Sahu,[1] Nagendra Kumar Koday,[3] Md Hasnat Ali,[2] Prashant Garg,[2] Swapna R Motukupally[2]

. *Br J Ophthalmol* 2015;**99**:1190–1195.

Fungal Keratitis



- Voriconazole inferior to Natamycin against *Fusarium* keratitis
- Both drugs equally effective against *Aspergillus* keratitis

Indian Journal of Medical Microbiology, (2015) 33(4): 538-546

Original Article

Ocular infections caused by *Candida* species: Type of species, *in vitro* susceptibility and treatment outcome

SR Motukupally, VR Nanapur, KN Chathoth, SI Murthy, RR Pappuru, A Mallick, *S Sharma

LVPEI
So that all may see

Fungal species	MIC 90 (µg/mL)					
	Amphotericin B	Voriconazole	Fluconazole	Natamycin	Caspofungin	Itraconazole
C albicans	0.25	0.125	3.0	8	0.38	2
C parapsilosis	0.50	0.5	24.0	4	0.75	3

Fungal Keratitis

Medical Management

Challenges

- Paucity of clinical trials
- Lack of interest in academia and industry
- Lack of experience with majority of antifungal agents
- Poor correlation with *in-vitro* activity

Antifungal activity of antibacterial antibiotics and preservatives

- * Occasional Reports of response of fungal keratitis to fluoroquinolones.
- @ Susceptibility of *Fusarium* and *Aspergillus* tested

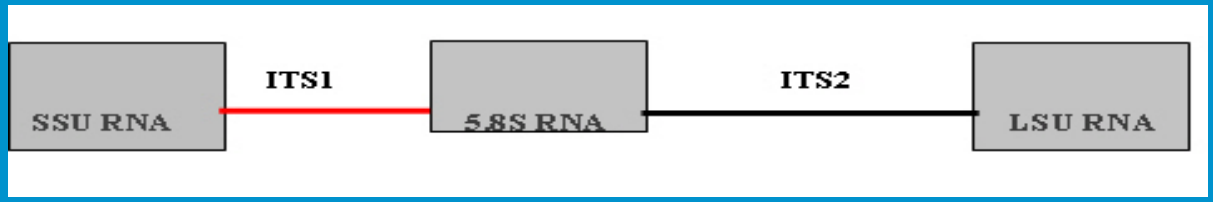
MIC 90- µg/ml

	Amox	Cefaz	Chloram	Moxi	Tobra	Benz
Fusarium spp.	>4000	>4000	2000	2000	700	64
Aspergillus spp.	>4000	>4000	4000	>4000	>4000	16

* Khor et al. JAMA 2006;295:2867
* Munir et al. Cornea 2007;26:621
@ Day et al.Br J Ophthalmol 2009;93:116

Investigation into hidden (non-sporulating) fungi

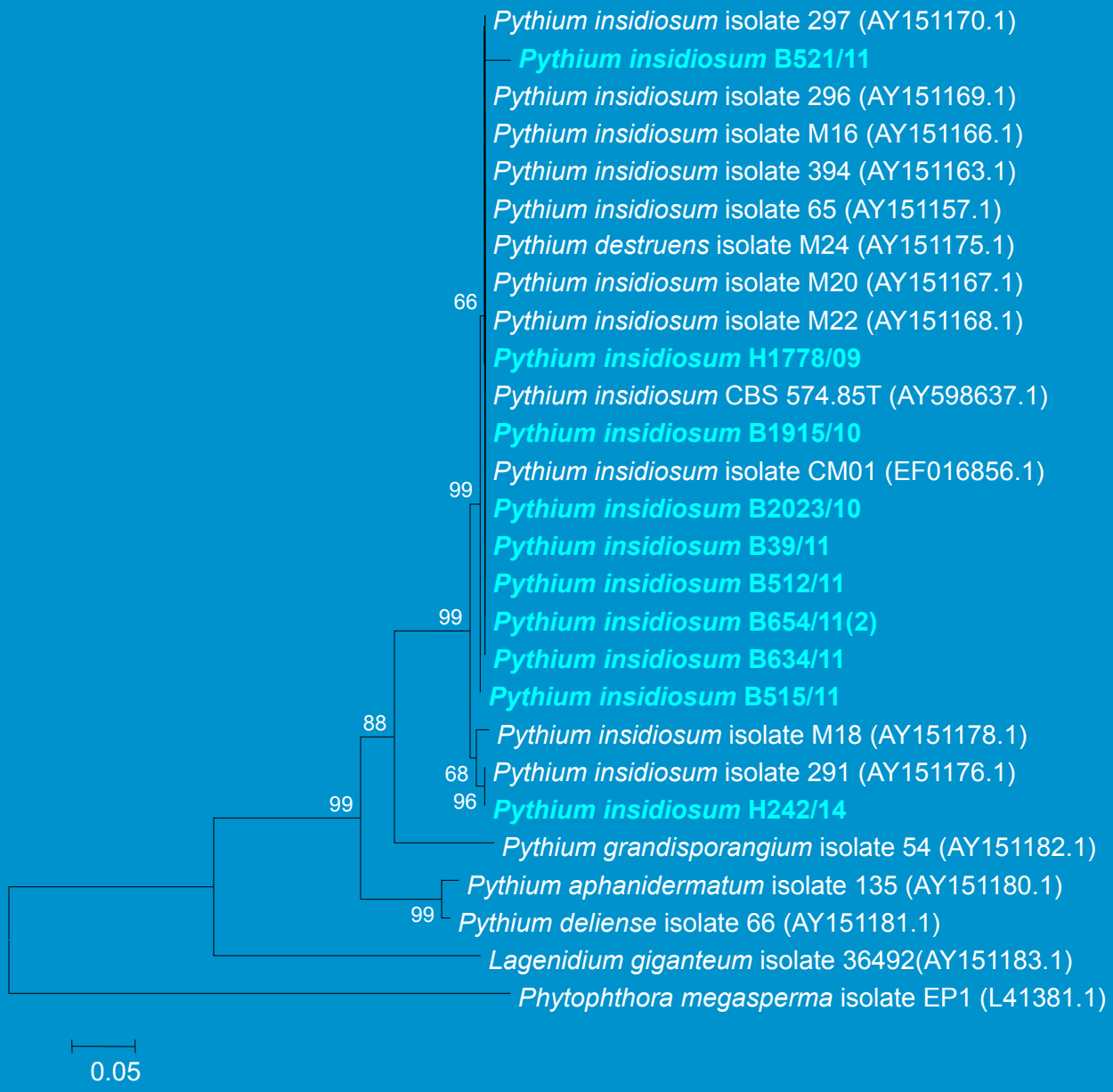
Many of the fungi grown from clinical samples remain unidentified due to lack of spores.

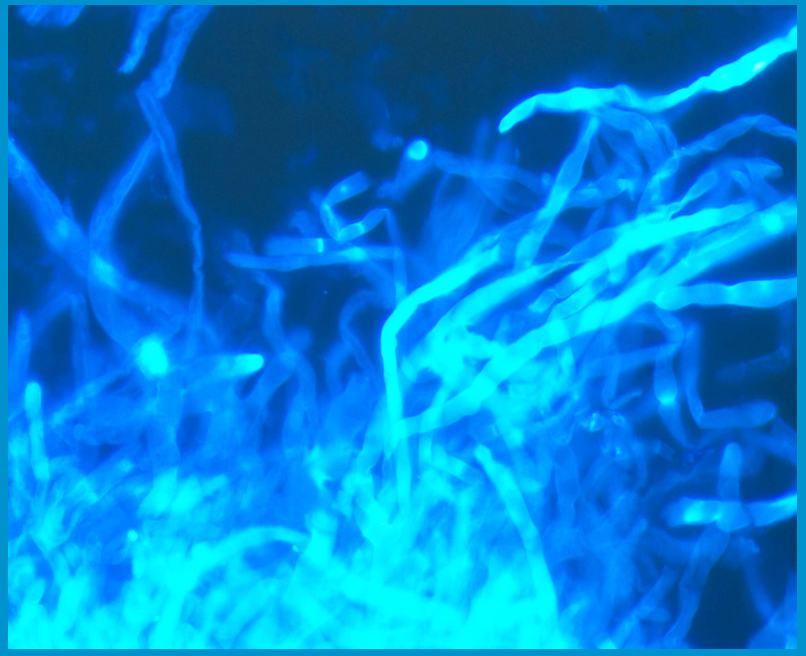


- Large number of ITS copies per cell (up to 250) makes the region an appealing target for sequencing samples.
- The entire ITS region ranges between 450 and 700 bp.
- Helps to find previously unknown species.

Gopinathan et al., *Cornea* 2002; 21(6): 555-559.

Srinivasn M. *Current Opinion in Ophthalmology* 2004; 15:321-327.


Pythium insidiosum isolate 297 (AY151170.1)
Pythium insidiosum B521/11
Pythium insidiosum isolate 296 (AY151169.1)
Pythium insidiosum isolate M16 (AY151166.1)
Pythium insidiosum isolate 394 (AY151163.1)
Pythium insidiosum isolate 65 (AY151157.1)
Pythium destruens isolate M24 (AY151175.1)
Pythium insidiosum isolate M20 (AY151167.1)
Pythium insidiosum isolate M22 (AY151168.1)
Pythium insidiosum H1778/09
Pythium insidiosum CBS 574.85T (AY598637.1)
Pythium insidiosum B1915/10
Pythium insidiosum isolate CM01 (EF016856.1)
Pythium insidiosum B2023/10
Pythium insidiosum B39/11
Pythium insidiosum B512/11
Pythium insidiosum B654/11(2)
Pythium insidiosum B634/11
Pythium insidiosum B515/11
Pythium insidiosum isolate M18 (AY151178.1)
Pythium insidiosum isolate 291 (AY151176.1)
Pythium insidiosum H242/14
Pythium grandisporangium isolate 54 (AY151182.1)
Pythium aphanidermatum isolate 135 (AY151180.1)
Pythium deliense isolate 66 (AY151181.1)
Lagenidium giganteum isolate 36492(AY151183.1)
Phytophthora megasperma isolate EP1 (L41381.1)
66
99
99
88
68
96
99
99
0.05

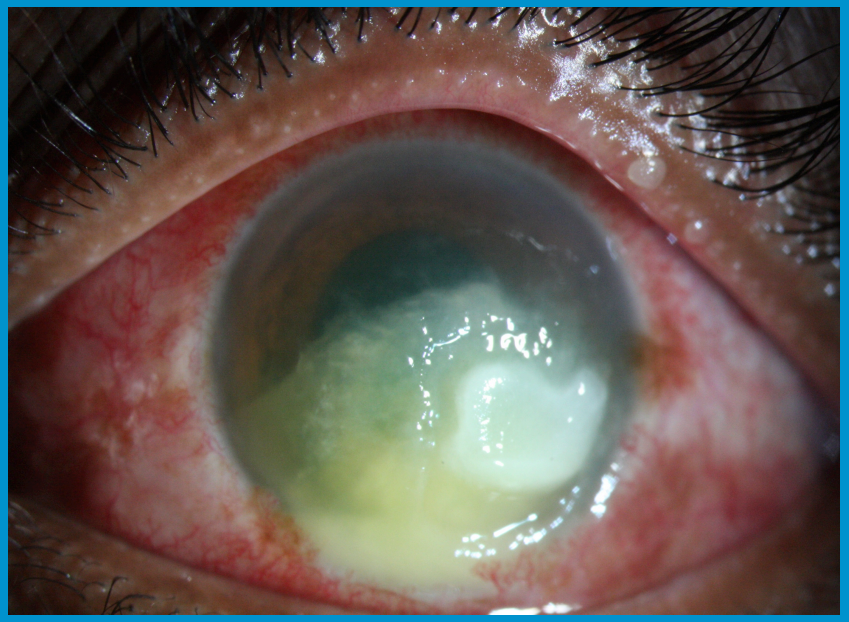


Broad, aseptate fungal filaments with ribbon-like folds
(KOH+CFW, x400)

Colourless, flat, appressed fungal colony with feathery edges
(Blood agar, 4 days growth at 37^{o}C)

Clinical Presentation of *Pythium* keratitis

Inferior stromal infiltrates with multiple linear tentacle-like infiltrates extending from superior edge of the limbus



Dry, full thickness infiltrates with hyphate edges and multiple dot-like infiltrates surrounding the main infiltrate

Report of 9 cases in 2010-12 (9/162, 5.5%); 4 cases in 2014 (4/102, 3.9%)
Sharma et al. *Pythium insidiosum* keratitis: Clinical profile and role of DNA sequencing and zoospore formation in diagnosis. Cornea 2015;34:438-442

Poor response to topical natamycin therapy

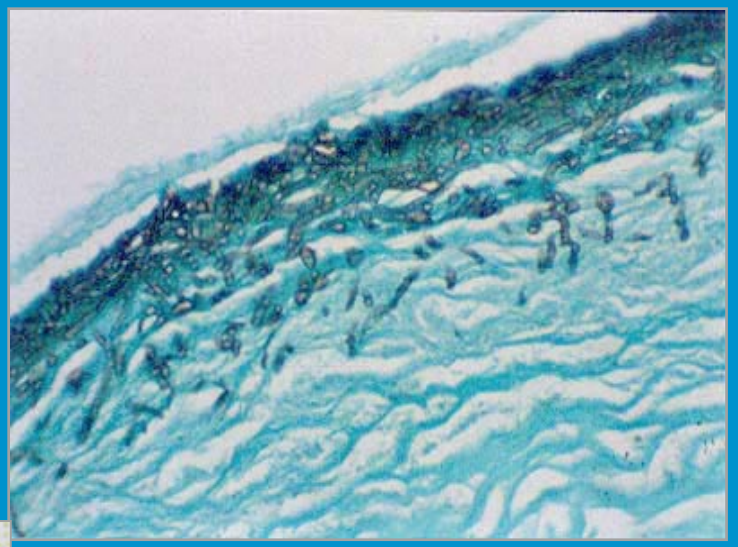
L V Prasad Eye Institute- Hyderabad data- 2014

(*Pythium insidiosm* keratitis- n=37)

- Prevalence- 37/728 (5%) fungal keratitis patients
- Mean age: 38.3± 13.8 years (range: 12-68 years)
- Males-17, Females-20
- Predisposing factors- trauma-15, none-22
- Dot like infiltrates-20, tentacles-6, ring infiltrate-6, plaque-6
- Corneal perforation -1
- Treatment- Topical natamycin, systemic antifungal-36, Intrastromal voriconazole-5, Collagen cross linking-1
- PK- 33, Recurrence after PK-8, failed graft-16

Surgical options for non-responding fungal keratitis

- Superficial keratectomy
- Tissue adhesive
- Lamellar Keratoplasty
- Penetrating Keratoplasty



Consequences of keratoplasty

- Long term medications
- Expensive
- Recurrence
- Graft Failure/Rejection
- Graft infections

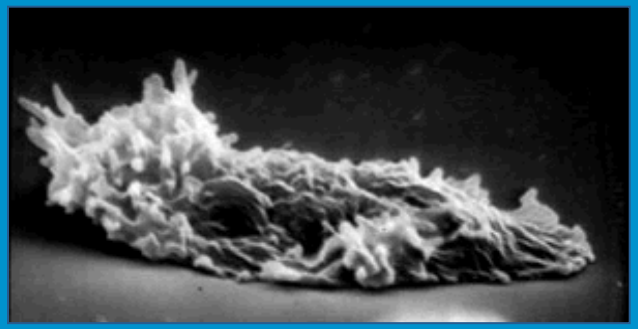
Efforts need to continue towards better medical therapy in fungal keratitis

Parasites causing eye infections

Protozoa	Helminths			Arthropods
	Nematodes	Cestodes	Trematodes	
Toxoplasmosis	Toxocariasis	Cysticercosis	Schistosomiasis	Ophthalmomyiasis
Acanthamoebiasis	Ascariasis	Echinococcosis	Paragonimiasis	
Entamoebiasis	Onchocerciasis		Coenurosis	
Malaria	Loiasis		Sparganosis	
Giardiasis	Dirofilariasis			
Leishmaniasis	Filariasis			
Trypanosomiasis	Dracunculiasis			
Pneumocystosis	Thelaziasis			
Microsporidiosis	Gnathostomiasis			
	Angiostrongyliasis			
	Trichinosis			

Acanthamoeba

- **Ubiquitous free living amoebae**
- **Chronic granulomatous encephalitis**
- **Keratitis**
  - **Inflammation of cornea**
  - **Significant monocular morbidity**
  - **First case diagnosed in 1973**
  - **In India - Madurai - 1987**
- **Disseminated cutaneous infection in AIDS patients**



Acanthamoeba Keratitis

- *Acanthamoeba* infection can mimic all types of keratitis: viral, bacterial or fungal
- It is frequently misdiagnosed as herpes simplex keratitis
- Mistakenly reported to be fungal keratitis in as high as 45% of the cases

Sharma S, Garg P, Rao GN. Patient characteristics, diagnosis and treatment of non-contact lens related Acanthamoeba keratitis. Br J Ophthalmol. 2000;84:1103–1108

Acanthamoeba keratitis

Conventional methods of diagnosis

	KOH/CFW		Gram		Giemsa		Total
	+	-	+	-	+	-	
Culture +	118	17	109	26	95	40	135 (81%)
Culture -	29	2	26	5	27	4	31
Total	147 (89%)	19	135 (81%)	31	122 (73%)	44	**166**

(Pasricha *et al*. *J Clin Microbiol* 2003)

Acanthamoeba Keratitis

Medical management

- Biguanides – Chlorhexidine 0.02%
  PHMB 0.02% to 0.06%
- Diamidines – Propamidine isethionate 0.1%
  Hexamidine 0.1%

Acanthamoeba Keratitis

Surgical management

- Epithelial debridement
- Amniotic membrane transplantation
- Lamellar keratectomy combined with conjunctival flap
- Phototherapeutic keratectomy
- Penetrating keratoplasty
- Deep lamellar keratoplasty

- Sharma S, Garg P, Rao GN. Patient characteristics, diagnosis and treatment of non-contact lens related *Acanthamoeba* keratitis. Br J Ophthalmol. 2000;84:1103–1108
- Parthasarathy A, Tan DT. Deep lamellar keratoplasty for *Acanthamoeba* keratitis. Cornea. 2007;26:1021–1023

Surgical treatment required by approximately 5-10% patients

Microsporidial infections of the eye

- Anecdotal reports in AIDS patients in 1990s
- Case reports of superficial keratoconjunctivitis in immunocompetent patients

  Lewis NL *et al*. *Cornea* 2003 : 374-6

  Moon SJ *et al*. *Cornea* 2003 : 271-2

- Ist report from India in 2003

  Sridhar *MS, Sharma S. AJO 2003 : 745-6*

(Routine smear examination of corneal scraping)
KOH+CFW, Gram stain, 1% AFS
Joseph et al. J Clin Microbiol 2006, 44:4

Diagnosis & Clinical Features

1: Ophthalmology. 2006 Feb 15; [Epub ahead of print]

ELSEVIER FULL-TEXT ARTICLE

Clinical and Microbiological Profile of Microsporidial Keratoconjunctivitis in Southern India.

Joseph J, Sridhar MS, Murthy S, Sharma S.

- **January 2002- December 2004, LVPEI-Hyderabad**
- **19 patients of microsporidial keratoconjunctivitis**

Diagnosis, clinical features and treatment outcome of microsporidial keratoconjunctivitis

Sujata Das, Savitri Sharma, Srikant K Sahu, Shyam S Nayak, Sarita Kar

Br J Ophthalmol 2012;96:793e795. doi:10.1136/bjophthalmol-2011-301227

- **March 2007-Oct 2010, LVPEI-Bhubaneswar**
- **270 patients of microsporidial keratoconjunctivitis**
- **Seasonal, diagnosed by simple lab. test, outcome satisfatory**

Treatment of Microsporidial Keratoconjunctivitis

Clinical Trial of 0.02% Polyhexamethylene Biguanide Versus Placebo in the Treatment of Microsporidial Keratoconjunctivitis

SUJATA DAS, SRIKANT K. SAHU, SAVITRI SHARMA, SHYAM SUNDAR NAYAK, AND SARITA KAR

Am J Ophthalmol 2010;150:110–115

CONCLUSIONS: Treatment of microsporidial keratoconjunctivitis with PHMB does not offer any significant advantage over placebo, suggesting self-limiting nature of the disease.

Microsporidial Stromal Keratitis

1: BMC Ophthalmol. 2005 Aug 17;5:19.

Full text free on... BioMed Central

FREE full text article in PubMed Central

Is microsporidial keratitis an emerging cause of stromal keratitis? A case series study.

Vemuganti GK, Garg P, Sharma S, Joseph J, Gopinathan U, Singh S.

- Five cases with DD - HSV /fungal/bacterial keratitis
- Steroids worsened the condition.
- Spores not seen beyond Descemet ‘s membrane in any case.
- Lack of effective medical therapy
- surgical treatment required

Microsporidial Stromal Keratitis

Intraocular Invasion by Microsporidial Spores in a Case of Stromal Keratitis.

S Das, Sharma S, Sahu SK, Vemuganti GK

Arch Ophthalmol/vol 129 (no. 4), Apr 2011

Currently 34 cases of stromal keratitis

Microsporidial Stromal Keratitis

Medical Management

- Fumagillin
- Albendazole
- Fluoroquinolones- Ciprofloxacin
- Biguanides- Chlorhexidine, PHMB

Poor response to medical management.
Keratoplasty is the rule rather than exception.

Summary

- Fungal/Parasitic Keratitis:
  - Suboptimal response to medical treatment
  - Antimicrobial resistance not well studied
  - More effective drugs and drug delivery systems required

Silencing Protease Targets in the Brain: Implications for Therapy of Alzheimer's Disease

Vijaya Gopal
CSIR-Centre for Cellular & Molecular Biology
Hyderabad

UK-India DBT-MRC workshop, 14-15 March 2016

Nucleic Acid Delivery

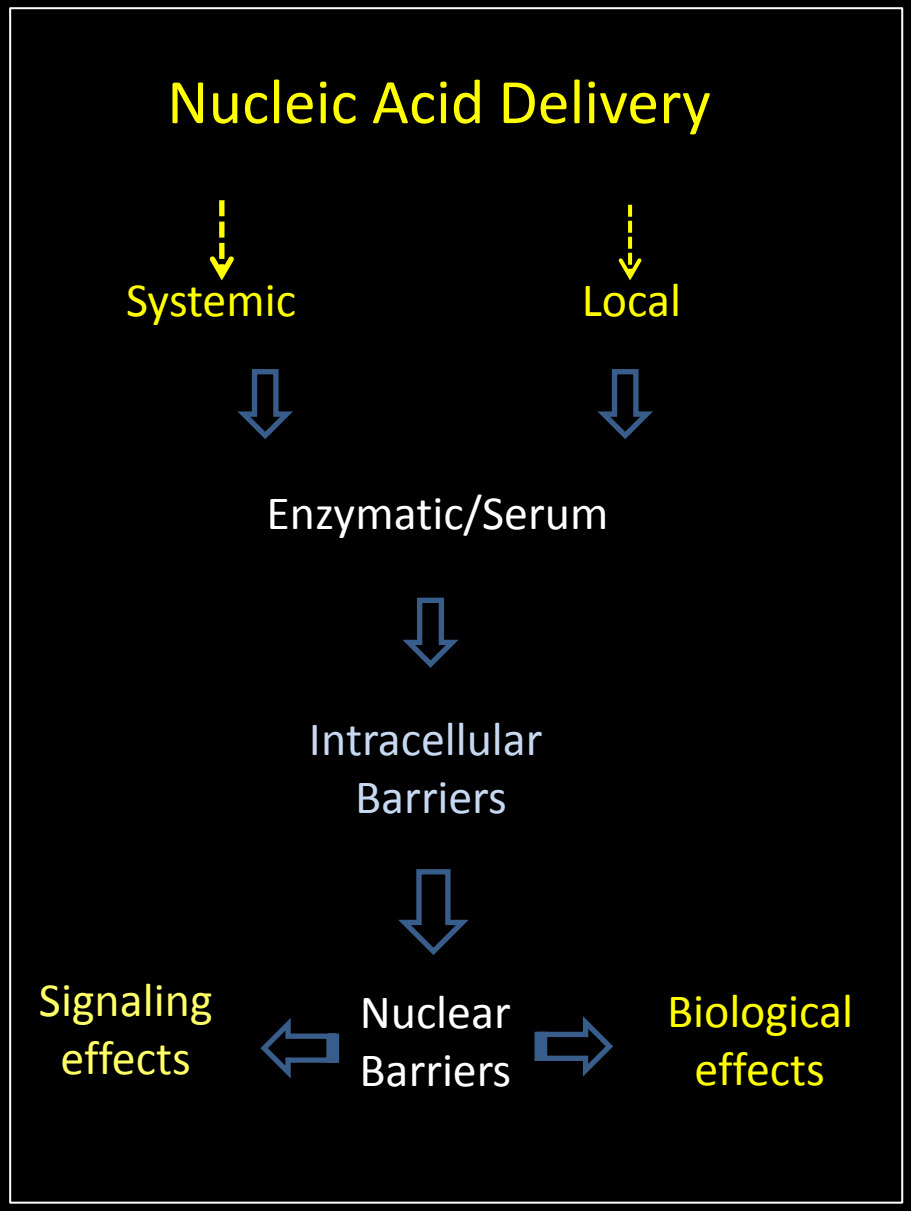
I RNAi therapeutics targeting neurodegenerative diseases

II Targeting HER2+ ovarian and breast cancer

III Development of multifunctional AuNP platforms for the co-delivery of nucleic acids and drugs

IV Structure-function activity of derivatized tocopherol-based formulations

V Lipid/peptide-mediated gene delivery



Peptide-guided gene delivery.
Martin & Rice, AAPS J. (2007)

Nature as a source of inspiration for cationic lipid synthesis.
Labas et al. *Genetica* (2010)

Peptides in DNA delivery: current insights and future directions. Mann et al. *Drug Discov Today* (2008)

Cationic liposomal lipids: From gene carriers to cell signaling. Lonez et al. *Progress in Lipid Research* (2008)

Rao & Gopal Cationic lipids for gene delivery in vitro and in vivo. *Expert Opinion on Therapeutic Patents* (2006)

Gopal, V Bioinspired peptides as versatile nucleic acid delivery platforms *J Control Release* (2013)

The promise of RNA Interference (RNAi)-Based Therapeutics

RNAi works via delivery of small RNA duplexes, including microRNA (miRNA) mimics, short interfering RNAs (siRNAs), short hairpin RNAs (shRNAs), and Dicer substrate RNAs (dsiRNAs)

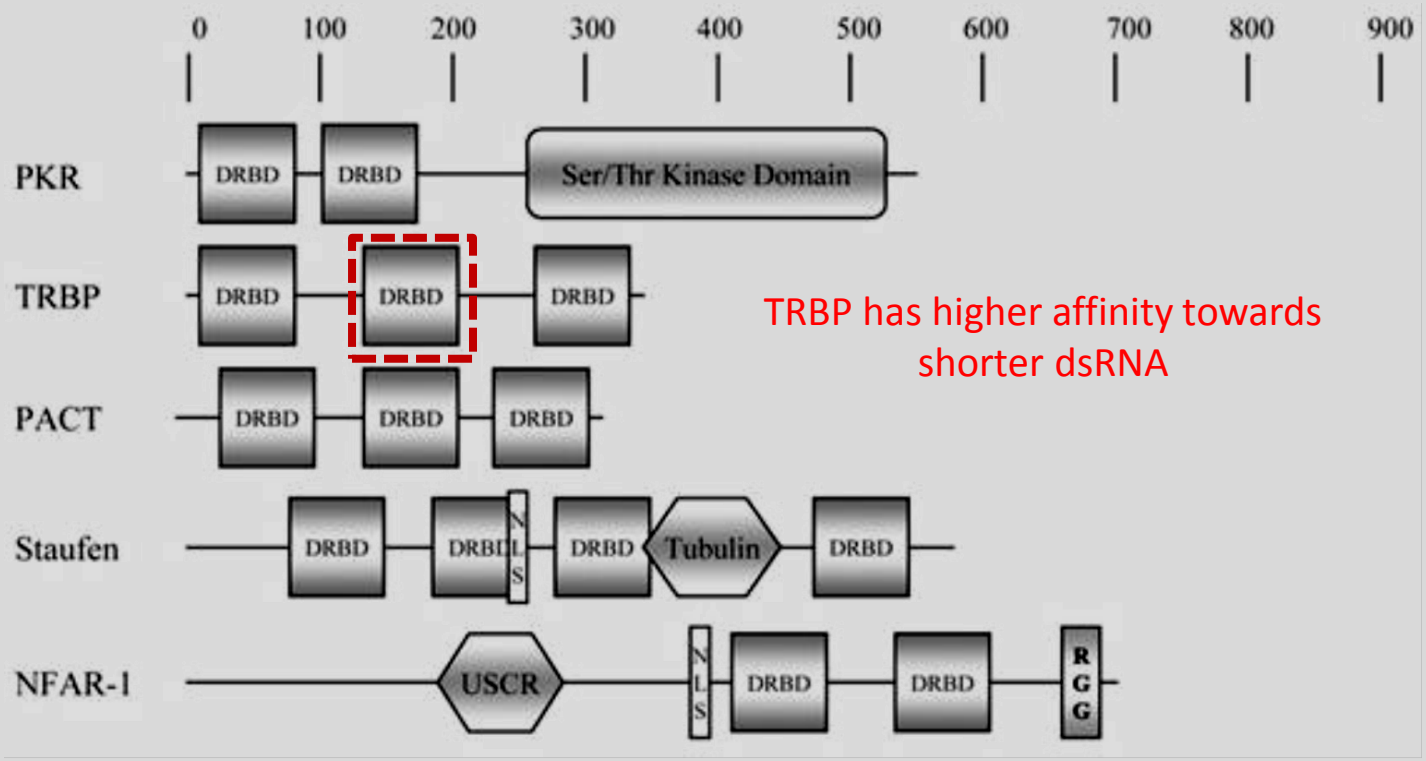
- dsiRNA-Dicer processing
- shRNA-nuclear processing
- siRNA and miRNA mimic pathways are the most direct (delivery to RISC loading)
- Unlike siRNAs, miRNAs are not 100% complementary to target sequences

Major considerations: Toxicity, Efficacy and Delivery

Key therapeutic advantages of RNAi

- Presence of RNAi machinery in mammalian cells
- High specificity and ability to evade drug resistance
- Potential of designing siRNA to any disease causing gene

Modular Structure of dsRNA binding proteins

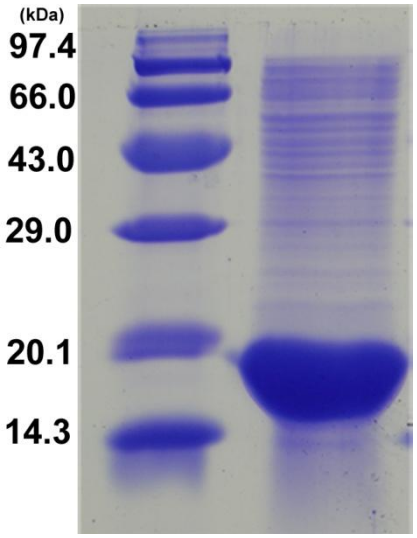


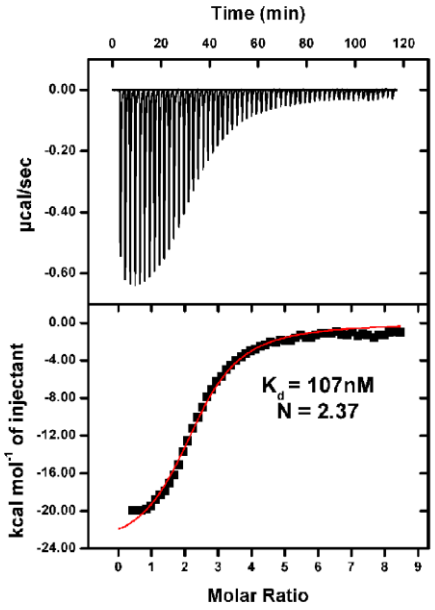
TRBP has higher affinity towards shorter dsRNA

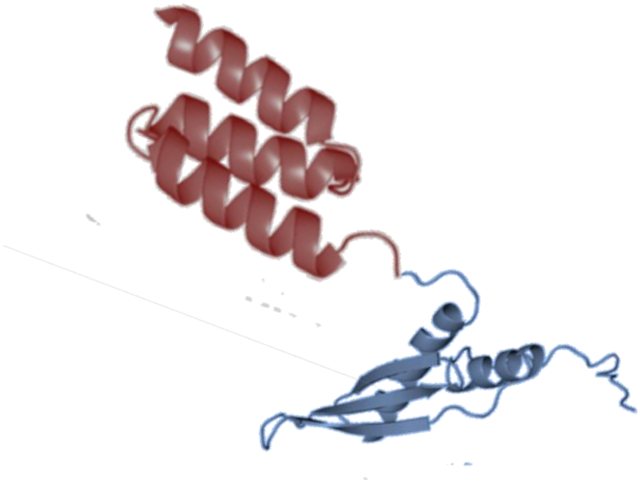
FASEB J (2003); *Nat. Rev. Cell Biol*. **(**2004)

Design and expression of recombinant fusion protein TRAF

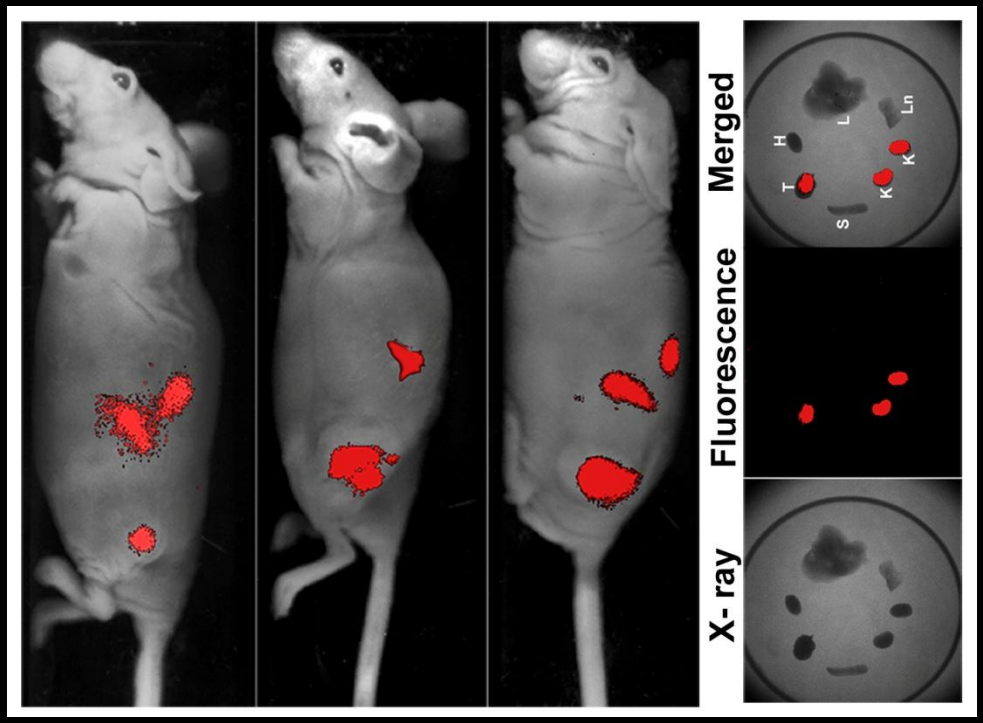
M.W = 18.6kDa



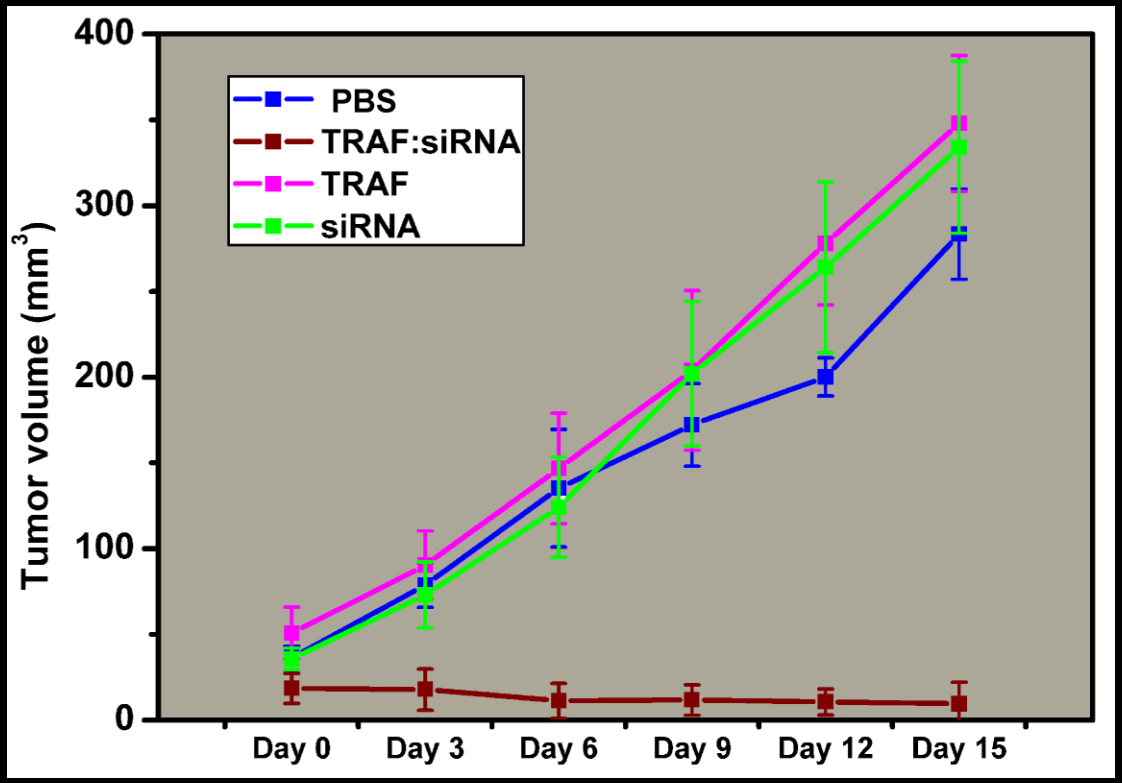




Selective accumulation of (TRAF:siRNA) into HER2+ tumor tissues



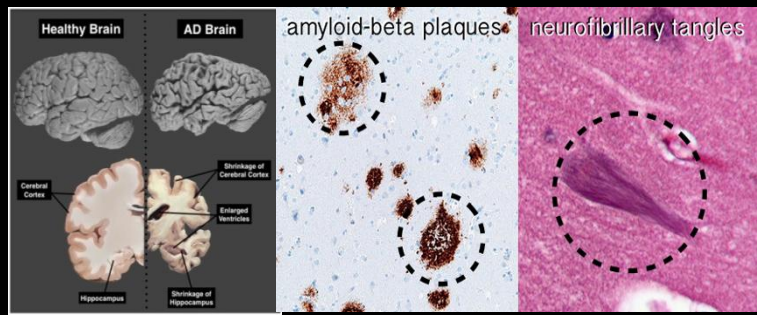
Systemic delivery of TRAF:siRNA complex led to significant suppression of tumor growth in xenograft mice



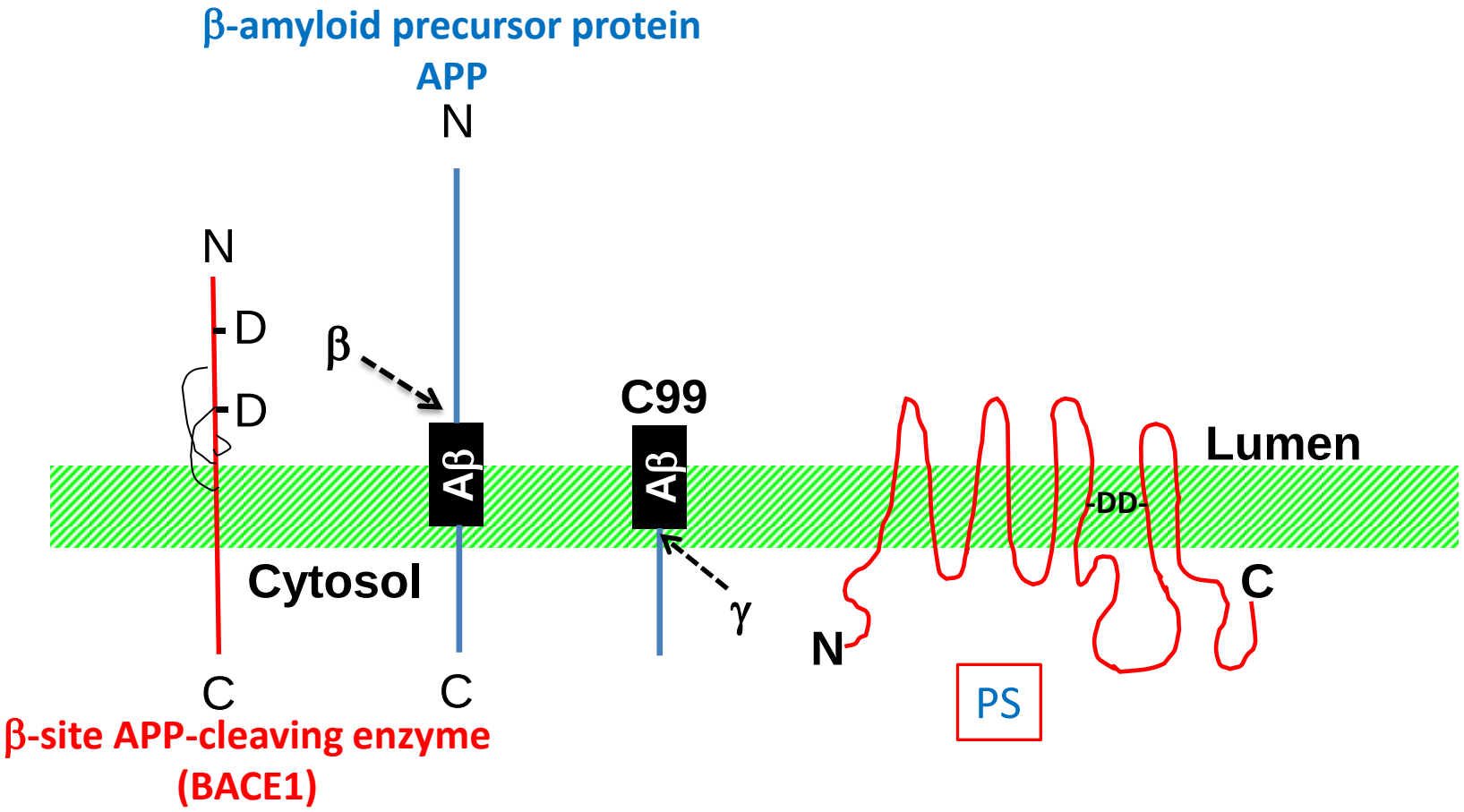
Dar et al. Nanomedicine (2015)

Aβ Generation

- Accumulation of amyloid-β is a **major signature** of Alzheimer's disease (AD).
- Formation of amyloid-β is catalyzed by γ-secretase, a protease with numerous substrates.
- Existing drugs for clinically effective γ-secretase inhibitors are toxic/mask symptoms but do not halt disease progression. Besides, these interfere with cellular homeostasis/are pumped out resulting in low efficacy and failures. (Svedruzik et al. PLOS ONE-8, 2013, Mangialasche et al. Lancet Neurol. 9, 2010, He et al. Nature, 2010).
- Key insights into molecular mechanism behind this complex disease is still being investigated to arrive at suitable therapeutic strategies. Targeting the enzyme complex, at the molecular level, to inhibit Aβ peptide production using RNA interference (RNAi) is potentially an attractive strategy for therapeutic intervention.
- However, the lack of efficient delivery systems is currently the bottleneck for clinical application of RNAi.



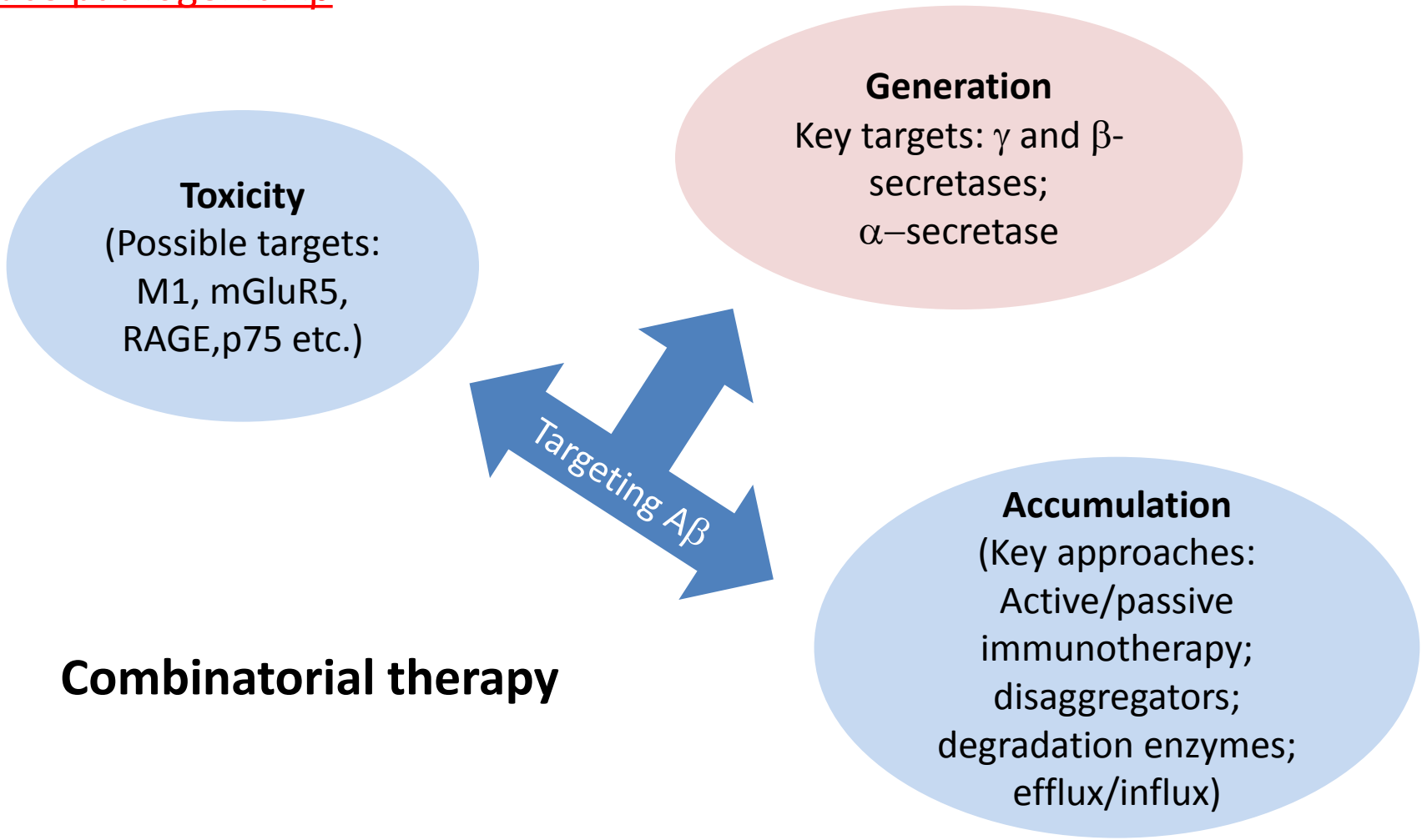
Aβ Generation



BACE1 initiates Aβ generation - a prime drug target for lowering cerebral Aβ levels in the treatment and/or prevention of AD

Various approaches targeting Aβ

Reduce pathogenic Aβ

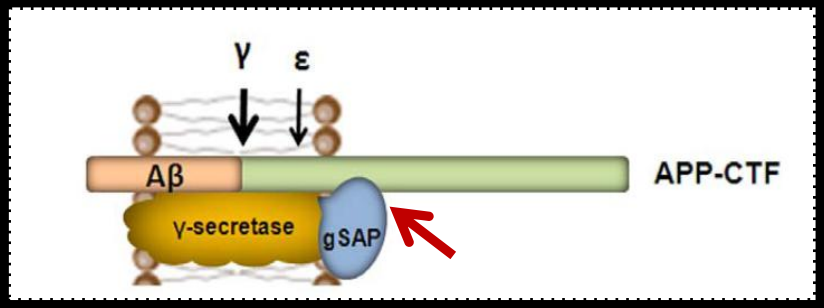


Combinatorial therapy

- Several approved drugs that reduce Aβ peptide by targeting γ-secretase also affect Notch processing, making them either toxic/less effective. Lack of specificity and weak potency of inhibitors has slowed down the development of promising drug candidates to this target.

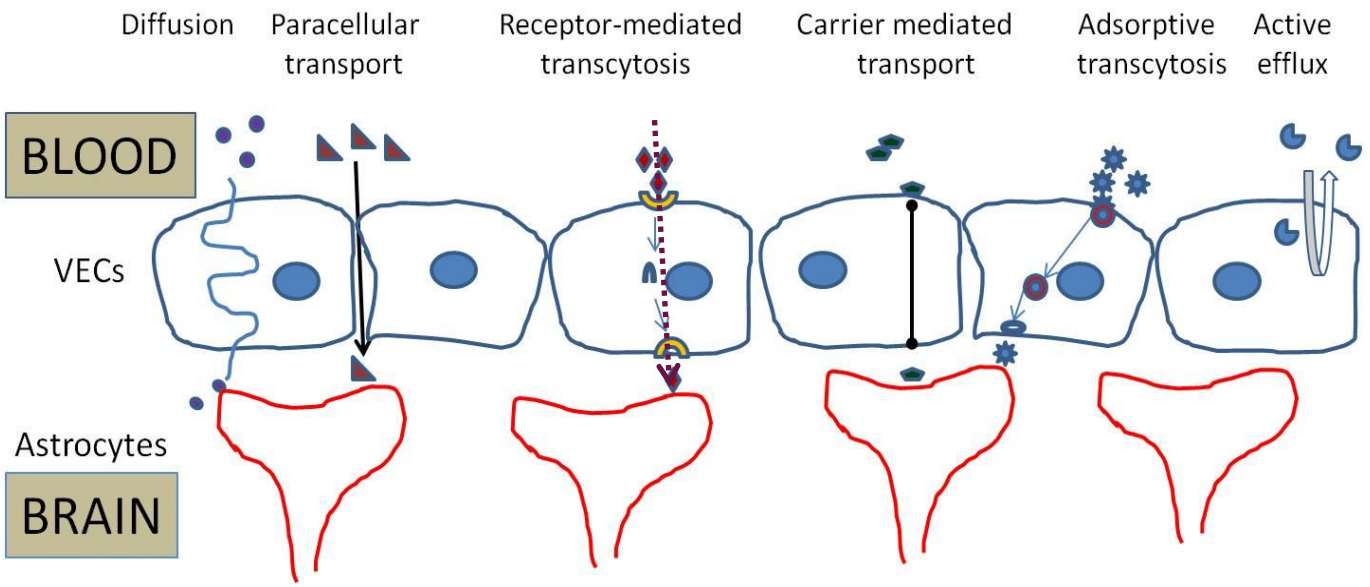
- The discovery of a γ-secretase activating protein (GSAP) that controls Aβ production suggests a potential and new molecular target that involves both γ-secretase and its substrate, the amyloid precursor protein C-terminal fragment (APP-CTF).

- Reducing GSAP was shown to decrease amyloid-β without affecting other key functions of γ-secretase.



(He et al. Nature 467, 95-98 2010)

Pathways for transport across the blood-brain barrier



Adapted from Abbott et al. *Nature Reviews|Neuroscience* (2006)

Current status for all RNAi-based therapies - 35 studies

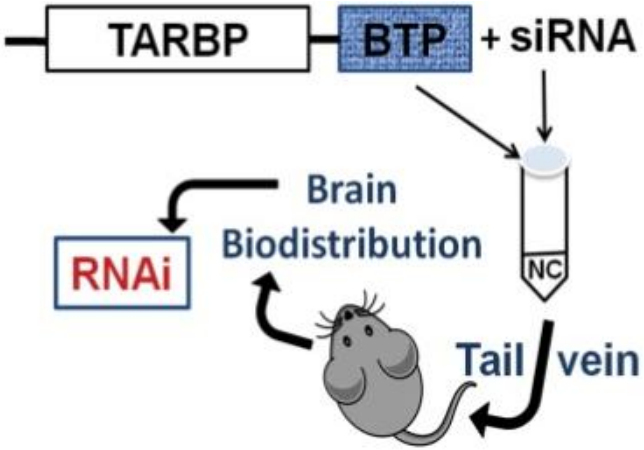
Viable neurons are targets for therapeutic intervention of AD

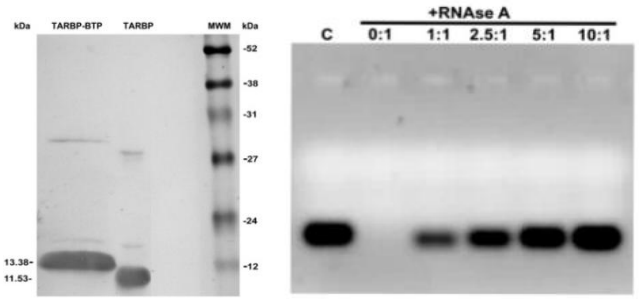
- More than 90 drugs are in various stages of clinical trials.
- NIH-Clinical trials - 1426 studies for Alzheimer's disease
- Gantenerumab, Memantine, Rivastigmine , NIC5-15, Varenicline, Donepezil, TRx0237, Interferon beta-1a, LY450139, ELND005, DAPT. http://www.clinicaltrials.gov/ct2/results?term=alzheimers&Search=Search
- **Status of vectors for receptor-mediated transcytosis to improve delivery of peptides into the brain:** Cationized albumin, transferrin, liposomes, mAbs to transferrin receptor, BDNF-OX26. Angiopep-2 peptide conjugated to paclitaxel- derived from ligands that bind LRP-1 highly expressed in the brain endothelial cells, neurons and astrocytes. T. J. Siahaan (Handbook of biologically active peptides - 2013).

In Alzheimer's disease, increased β–secretase(BACE1) activity has been associated with neurodegeneration and accumulation of amyloid precursor protein (APP) products. Inactivation of BACE1 could be important in the treatment of Alzheimer's disease.

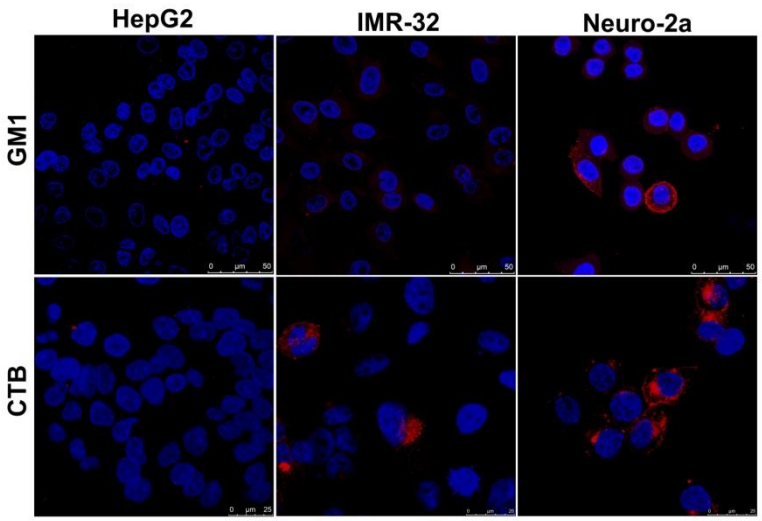
β–Secretase (BACE 1)	Lentiviral vector	Singer et al. *Nature Neuro.* 2005
Acetylcholine receptor	RVG-9R	Kumar et al. Nature, 2007
γ-secretase activating protein	Therapeutic target	He et al. *Nature*, 467, 2010
β–Secretase (BACE1) via GM1	TARBP-BTP (13.5kDa)	Haroon et al. *J Controlled Release* 2016

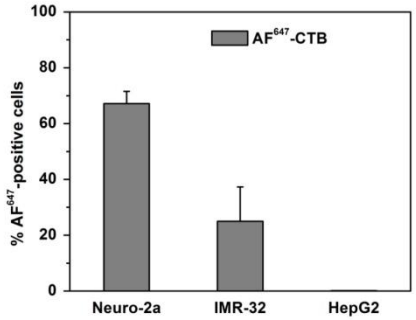
Construct design and *in vitro* analysis of TARBP-BTP fusion protein





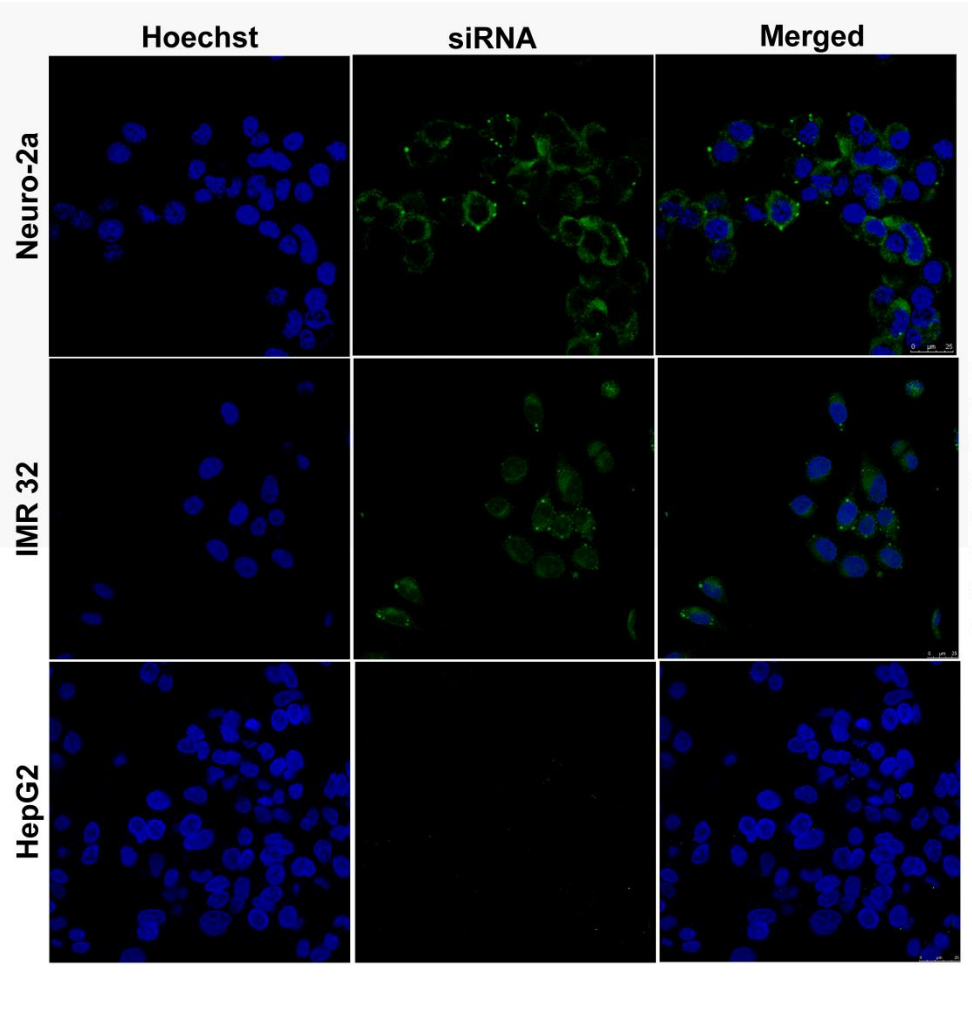
Determination of GM1 levels

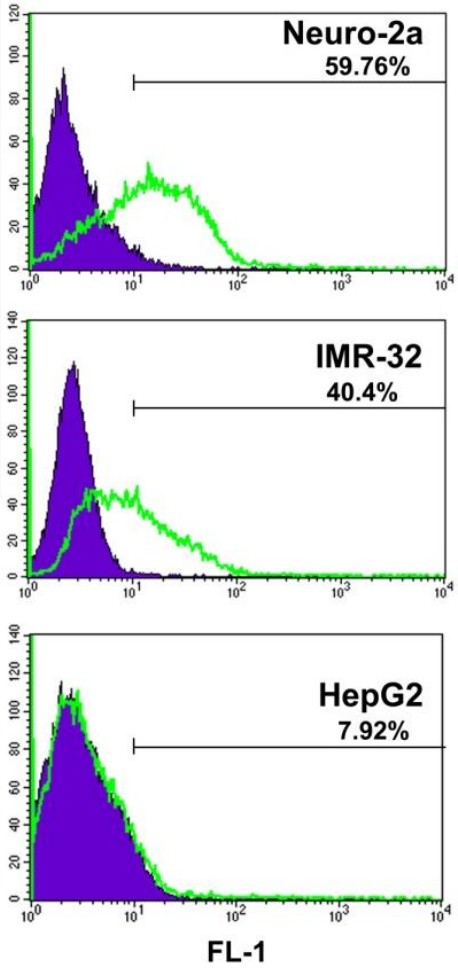




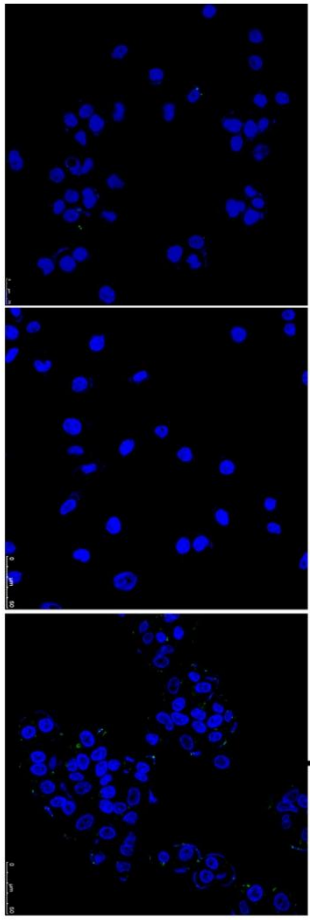
Alexa Fluor^{647}-CTB

Uptake of TARBP-BTP: siRNA complex





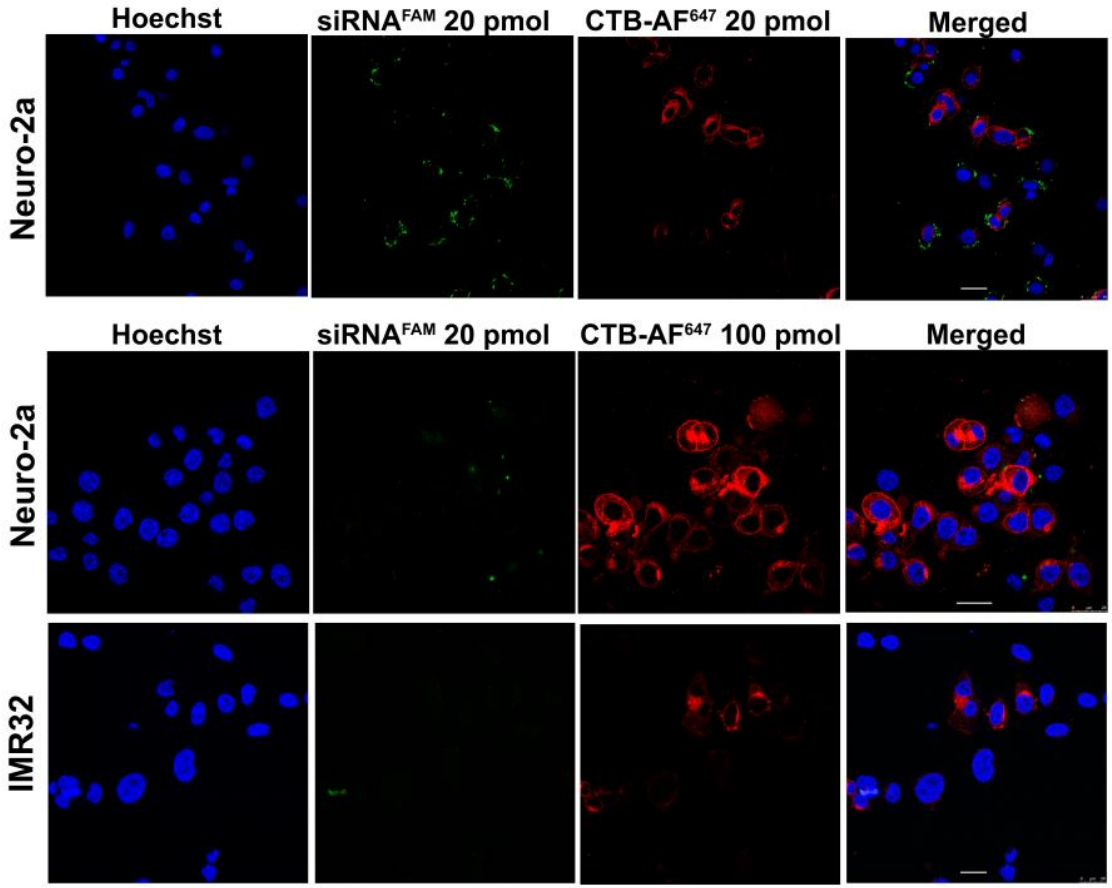
TARBP: siRNA



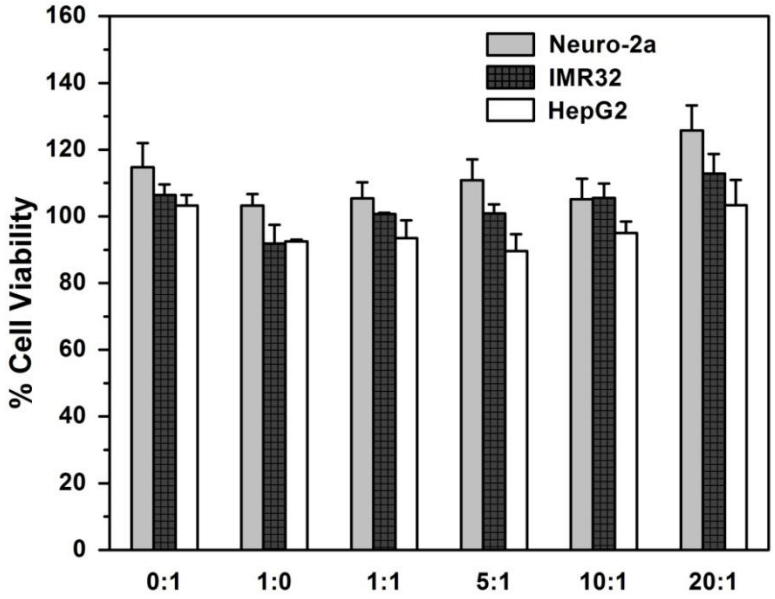
TARBP-BTP mediated recognition of Ganglioside GM1

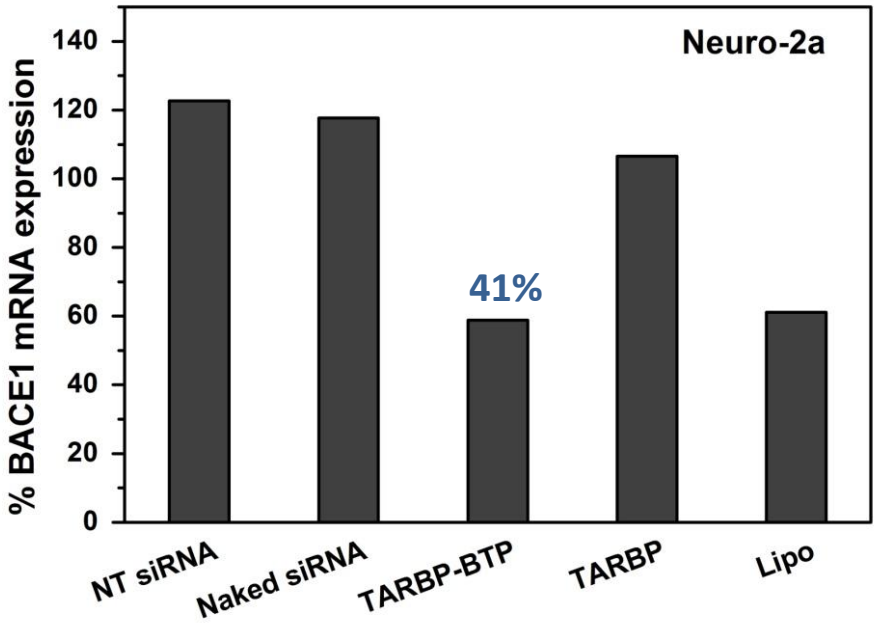
Carrier w/o the targeting ligand

TARBP-BTP recognizes ganglioside GM1



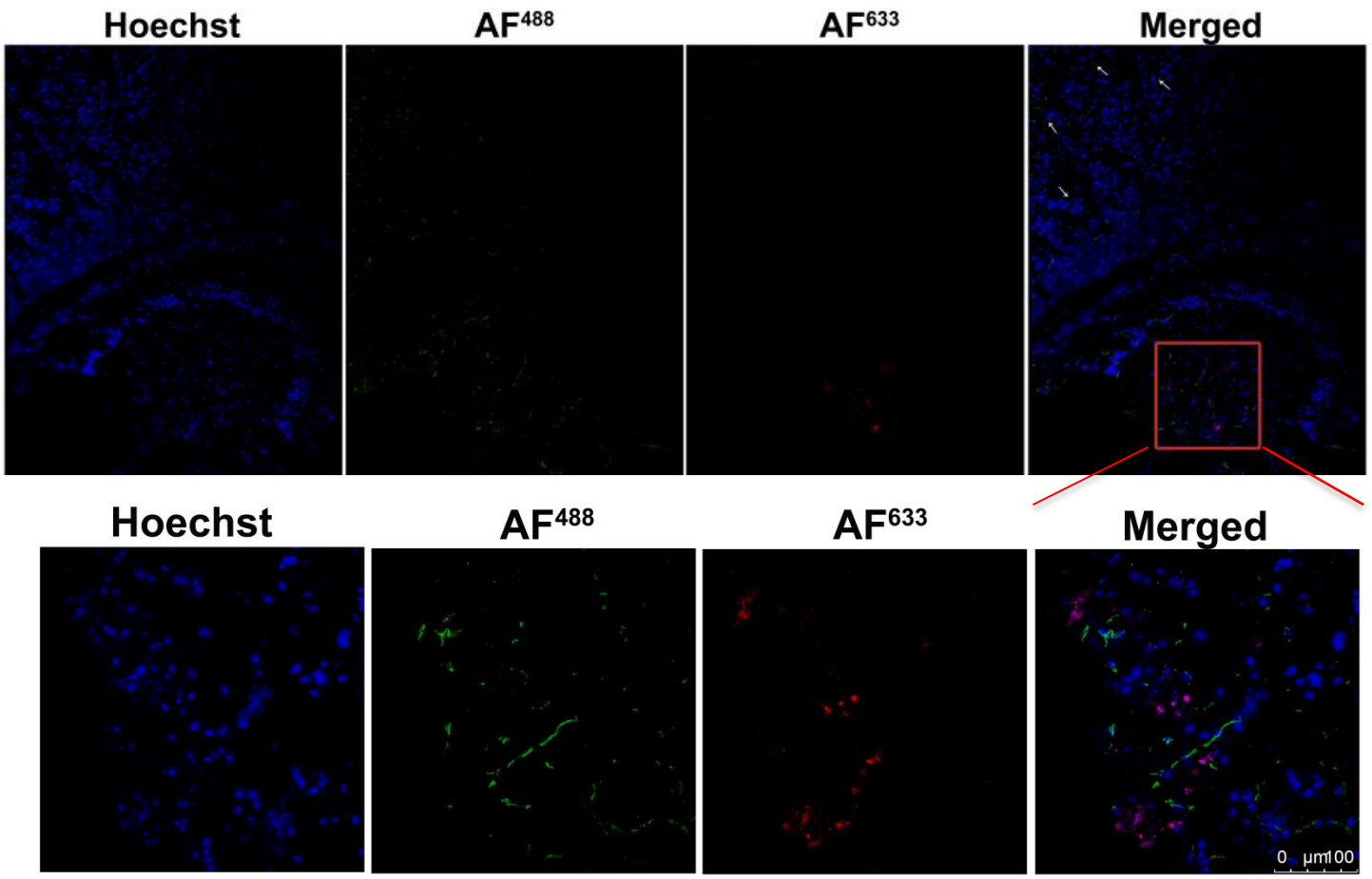
TARBP-BTP fusion protein is non-toxic





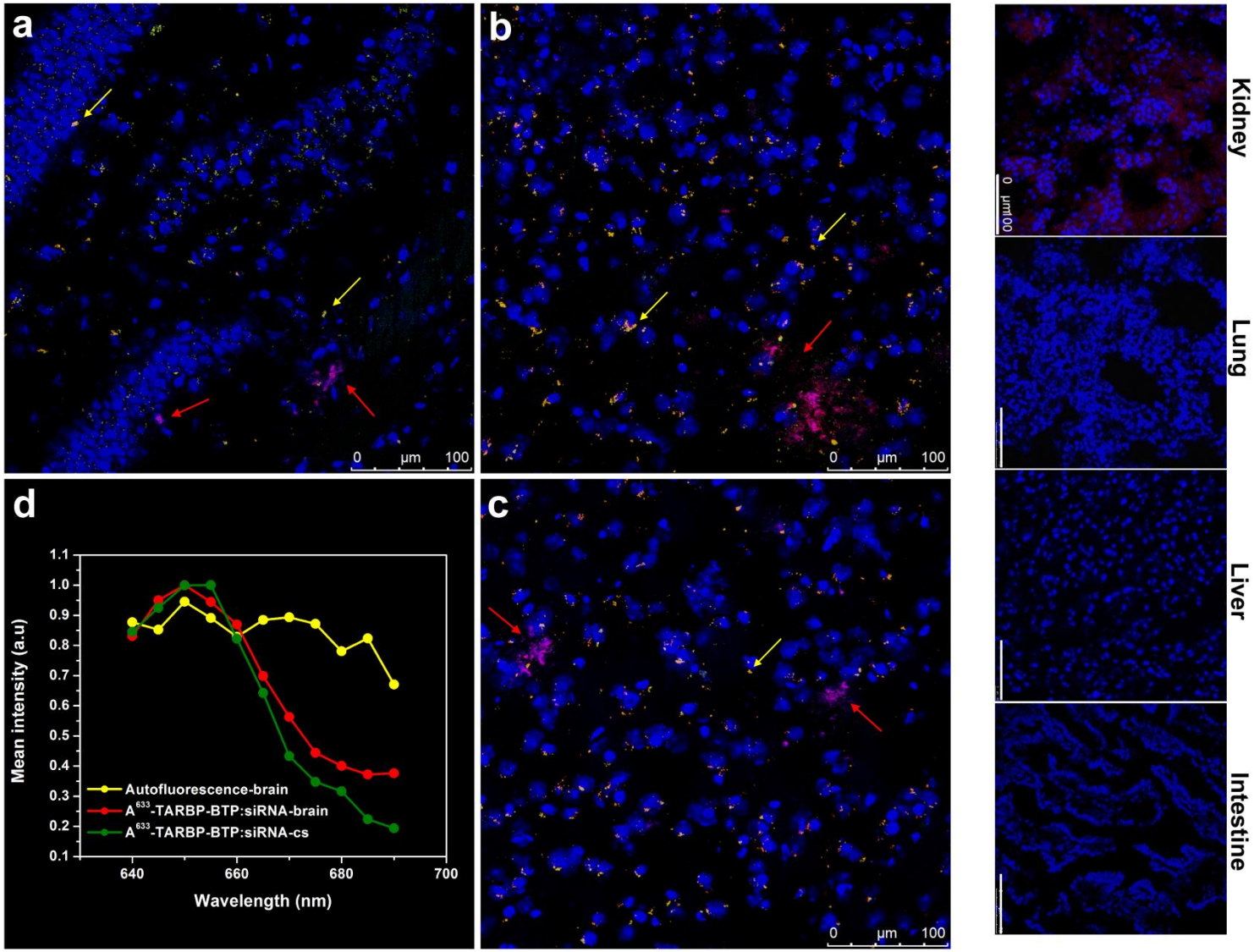
TARBP-BTP mediated knockdown of BACE1

Biodistribution of TARBP-BTP complex in AβPP-PS1 mouse brain

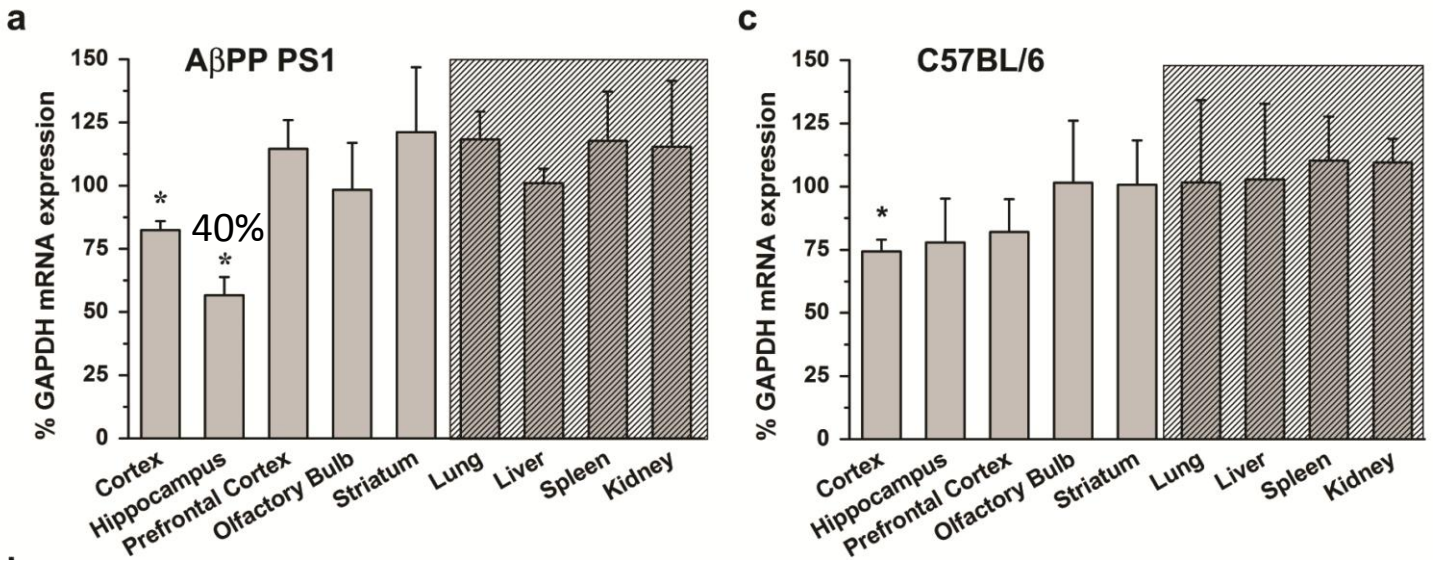


Target specificity

Localization of TARBP-BTP complex in the brain

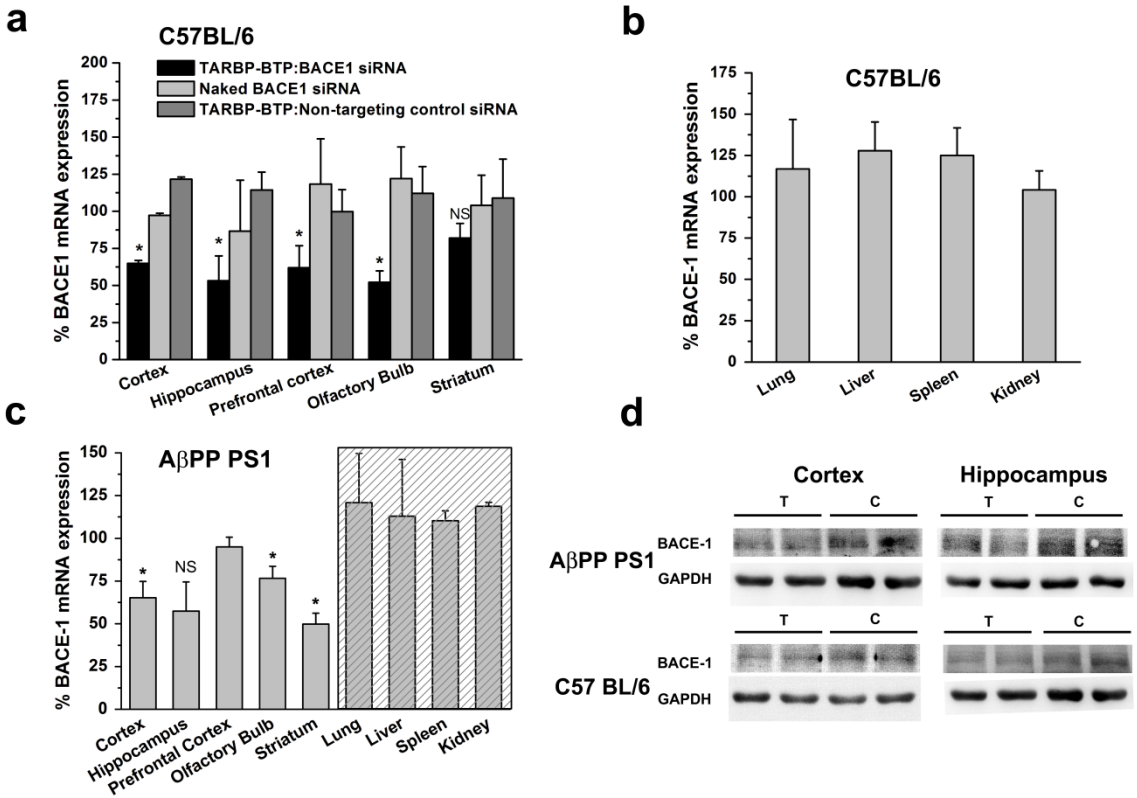


Silencing of endogenous GAPDH



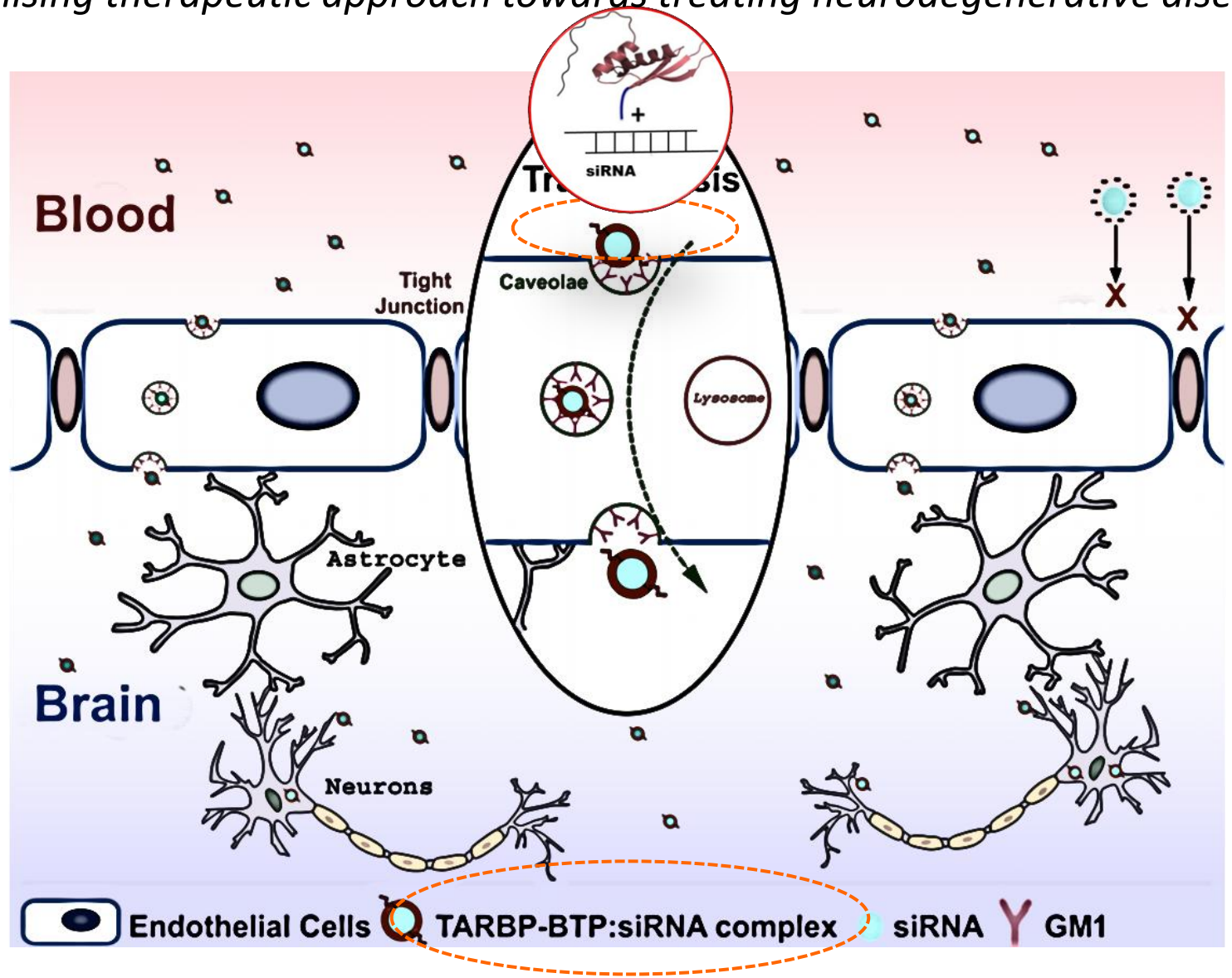
TARBP-BTP mediates RNAi of GAPDH in the hippocampus and cortical regions

TARBP-BTP-mediated gene silencing of BACE1 in APPswe-PS1dE9 (AβPP-PS1) AD mouse model



Targeted delivery of BACE1 siRNA results in 30-50% reduction in BACE1 expression
Haroon et al. *J Controlled Release (*2016)

A promising therapeutic approach towards treating neurodegenerative diseases!



Outcome of a combinatorial therapy targeting GSAP and BACE1?
Reduction in Aβ levels?

The potential for RNAi therapeutics is limited by delivery technology

Universal non-viral RNAi delivery systems for Ocular Diseases

Targeted manipulation of gene expression

- RNAi is promising and rapidly moving in the translational research space.
- The specificity of silencing virtually any gene by design of peptide-based delivery systems may offer the possibility of surpassing limitations of the BAB/BRB to deliver siRNA without side effects.
- Alternative to issues pertaining to drug resistance

- Duvvuri et al. *Expert Opin Biol Ther* 2003, 3: 45-56
- Del Amo EM, Urtti A*Drug DiscovToday* 2008, 13: 135-143.
- Li et al. Diagn Pathol. 2009; 4: 46.
- Thakur et al. *Journal of Biological Engineering*20126:7
- Reischl and Zimmer, *Nanomedicine: Nanotechnology, Biology and Medicine* 2009, 5: 8-20.
- Bobbin & John Rossi, Annu. Rev. Pharmacol. Toxicol. 2016. 56:103–22

Future scope

- Ocular disease is an easy target for RNA-based therapeutics.
- Disease targets and RNAi drug candidates in clinical trials

Approved RNA therapeutics

-Macugen –Targets VEGF in the eye

Other targets include the B2 adrenergic receptor, RTP801, and TRPV1.

Bevasiranib (NCT00499590), an anti- VEGF siRNA therapy for wet macular degeneration was discontinued during Phase III trials

-SYL040012, or bamosiran, is the second RNAi therapeutic that Sylentis has in clinical development. This RNAi therapeutic is currently in a Phase IIb clinical trial for patients with glaucoma, ocular hypertension, or open-angle glaucoma.

-Caspase-2, a protein involved in apoptosis to reduce retinal ganglion apoptosis

These diseases are caused by degeneration of the optic nerve, which leads to irreversible visual field loss

Future challenges should encompass strategies for delivery to less accessible areas to limit off-target effects

Acknowledgements

Group members
Mohamed Haroon
Ghulam Hassan Dar
Uthra Venkatraman
Durga Jeyalakshmi
Thasneem Yousuf
Aabid Shah

Collaborations
Anant Patel (CCMB)
N. M. Rao (CCMB)
Chittaranjan Patra (IICT)
Srilakshmi (NIT-Warangal)
Mangal Nagarsenkar (Mumbai College of Pharmacy)

Support

NOVEL POTENTIAL CARRIERS FOR HYDROPHOBIC DRUGS

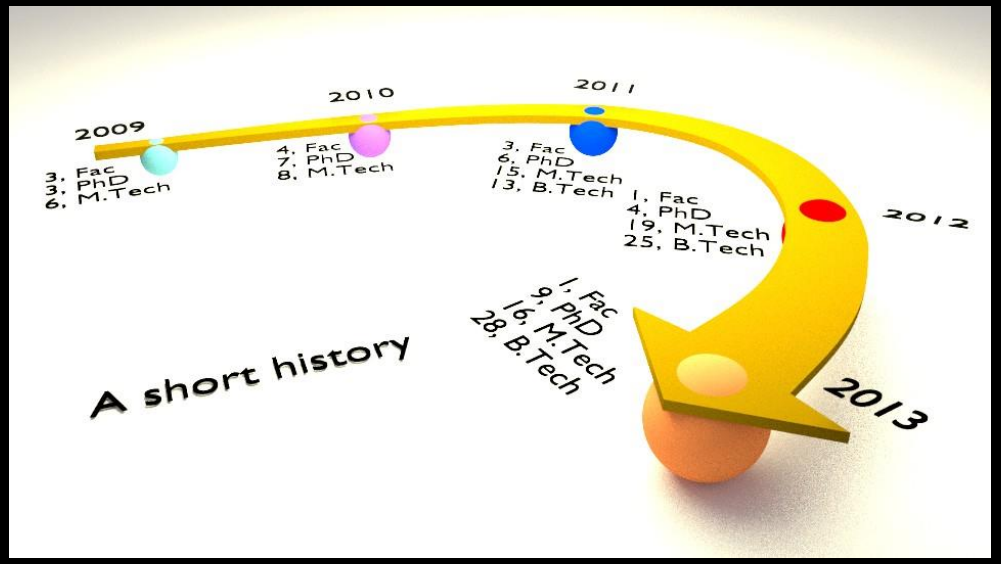
Utkarsh Bhutani & Saptarshi Majumdar

Department of Chemical Engineering

IITH: A New IIT with New Dreams

- Biggest among new IITs: 1900+ students
- 1:1 UG:PG Ratio: Focus on Research
- 100 cr.+ Funded Projects
- 1000+ Research Publications
- 160+ faculty; 13 branches; Fractals
- ~600 acres campus

Dept. of Chem. Engg.



17 faculty

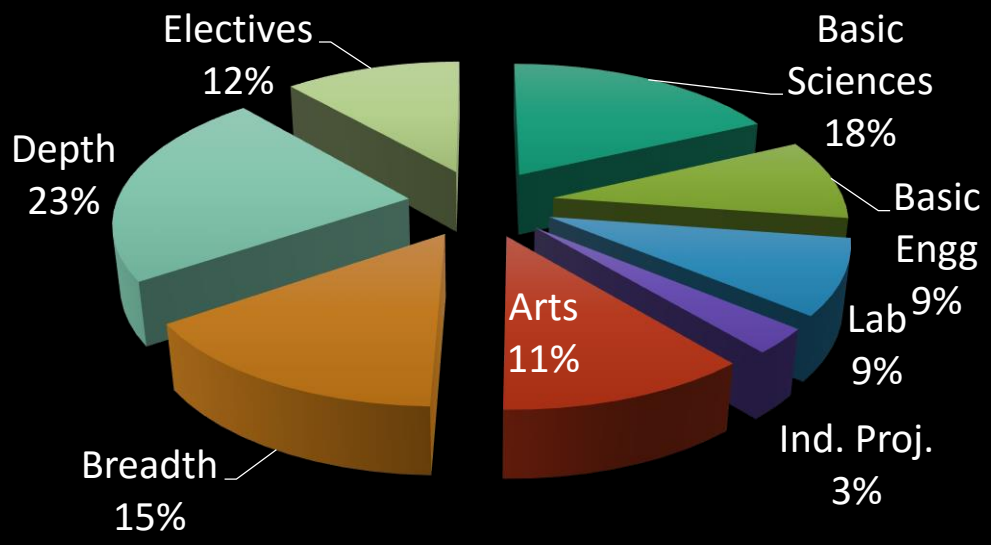
~200 students

B.Tech, M.Tech, Ph.D

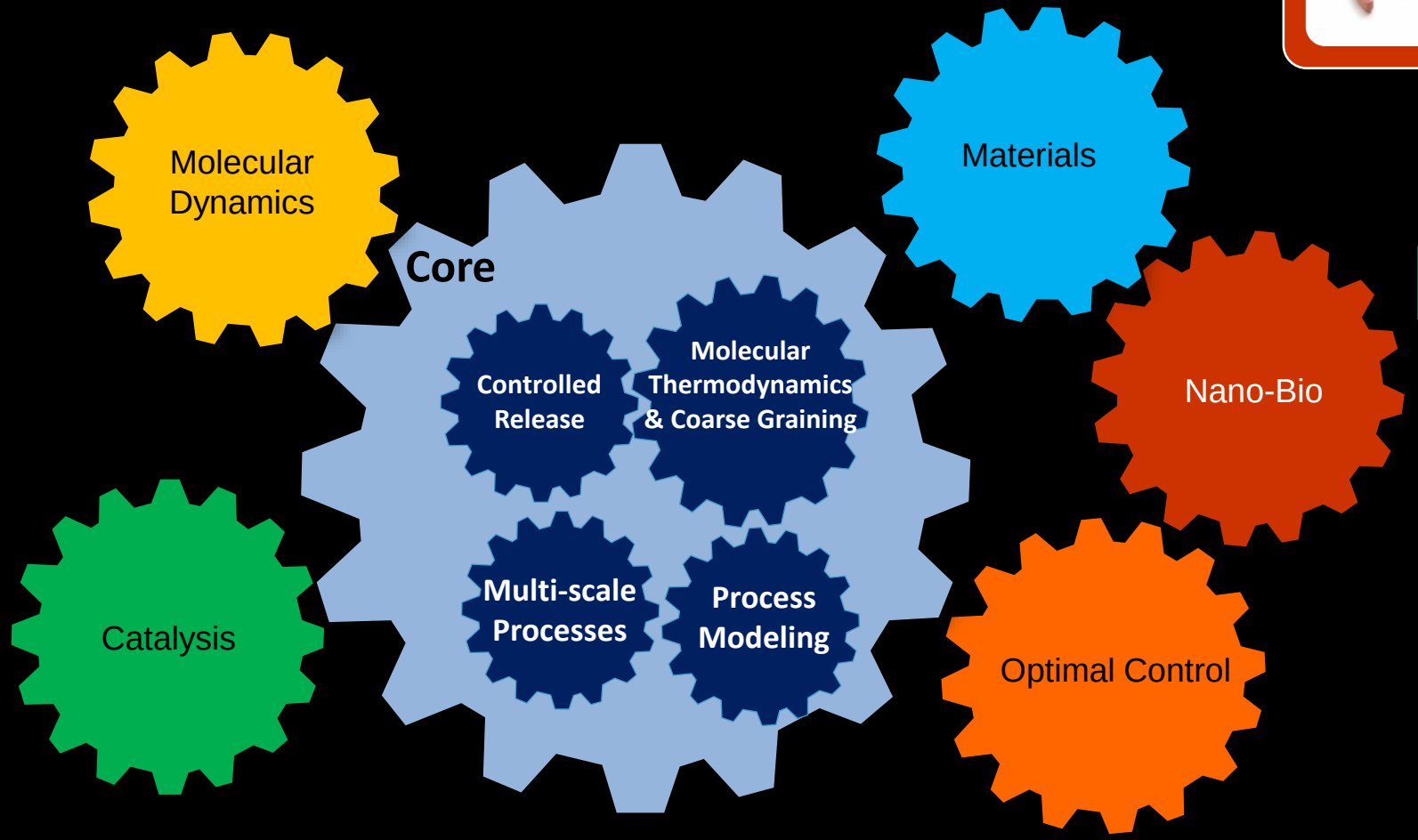
State of the art research facility

Many publications, funded projects, patents and awards

Nano, Poly, Bio, CFD, Reactions, Controls, Modeling, Optimization, Energy, Minerals



Current Engagements & Collaborations



Drug Delivery Systems (DDS)

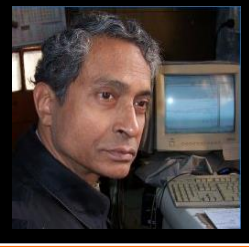
- Polymer, Biodegradable, Swelling, Morphology, Stability, Porosity, Diffusion, Release, Enzyme, pH

Dr. C S Sharma, IITH

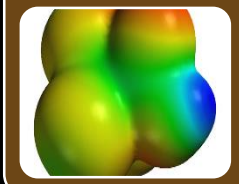
Dr. D Shee, IITH

Dr. K Mitra, IITH

Dr. D Chaudhuri, IOP

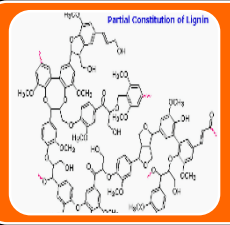


Prof. P Ray, CU



Multiscale Modeling

- Thermodynamics, Liquid Models, Coarse Graining (CG), Molecular Modeling, Dissipative Particle Dynamics -DPD

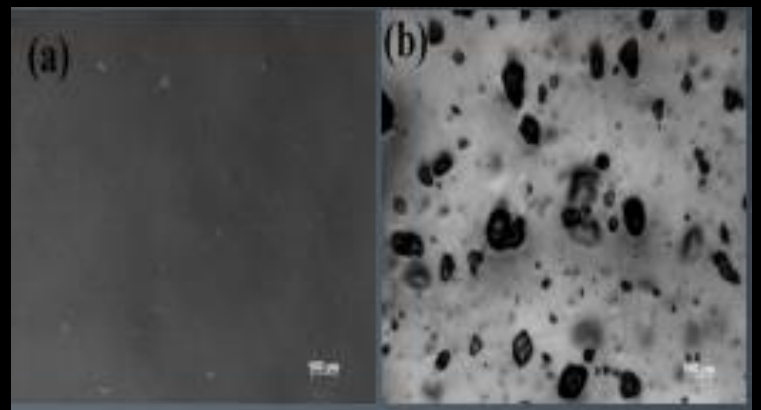


Lignin to BTX/ Lower Phenolics

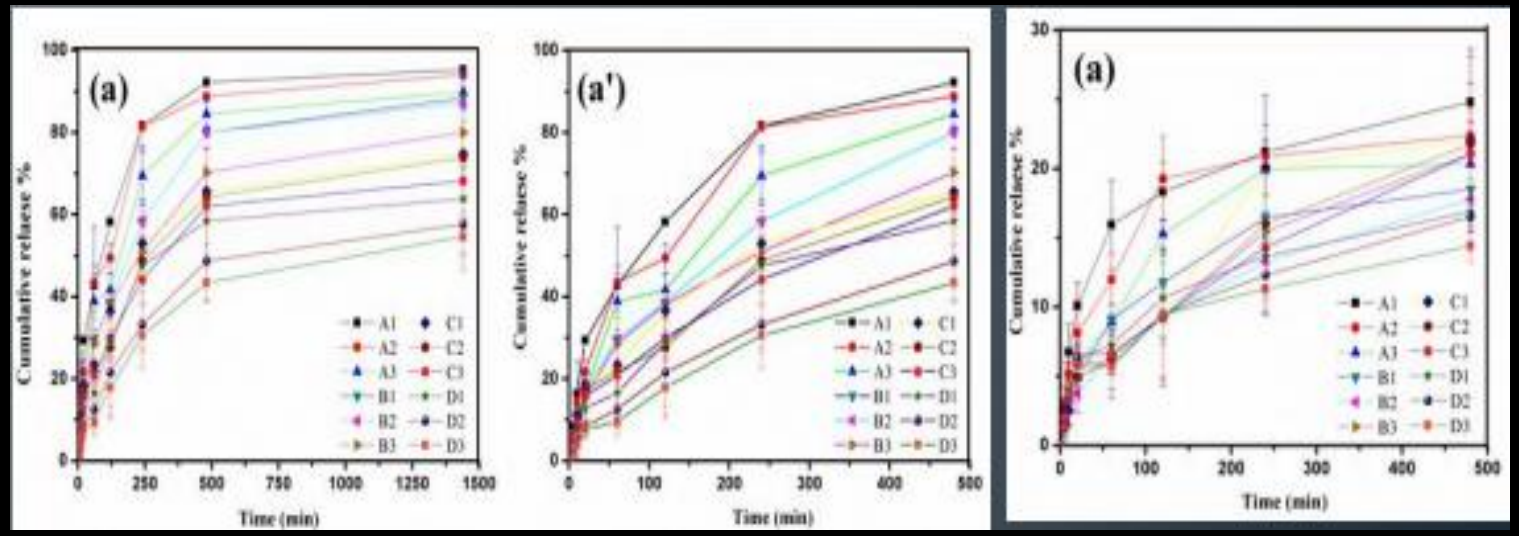
- Lignin, Degradation, Catalysis, BTX, Lower Phenolics, Kinetics, Solvent, Liquid Products

Gelatin Casted Thin-Film Based DDS

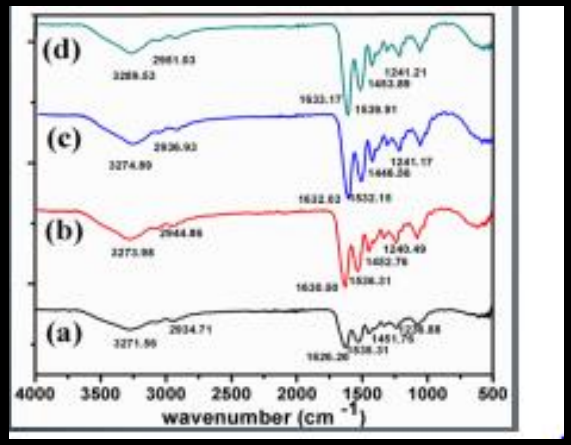
Anindita, Ph.D Student



Optical microscopic image of (a) without drug gelatin film, (b) drug loaded gelatin film.



Cumulative release of Piperine for different cross-linking polymeric film in pH 7.4 and 1.2 for 24h and 8h

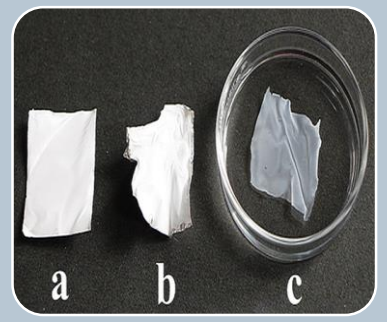


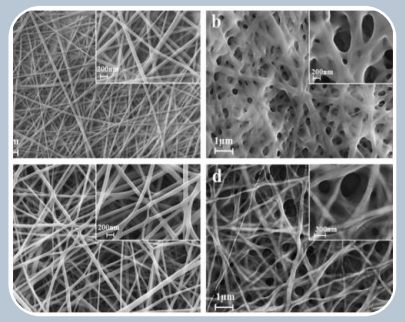
ATR/FTIR spectra for (a) NC, (b) cross-linked (0.1%v/v GTA) gelatin film (15%w/v); (c) NC(Piperine loaded), (d) cross-linked (0.1%v/v GTA and Piperine loaded) gelatin film (15%w/v).

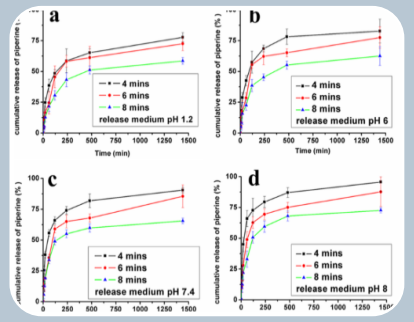
pH of solution	A=2.5% w/v gelatin casted film and cross-linking time= 10min t=0.0518±0.005418 mm						
	A0 (0%v/v)	A1 (0.01 %v/v)	A2 (0.02 %v/v)	A3 (0.05 %v/v)	A4 (0.10 %v/v)	A5 (0.25 %v/v)	Main cause of drug release
1.2	-	+	+	*	*	*	Degradation
6	-	+	+	*	*	*	Degradation
7.4	-	+	+	*	*	*	Degradation
8	-	+	+	*	*	*	Degradation

Materials and Manufacturing Processes, 2016, 31, 223-230

Controlled Drug Delivery: Nano-fiber DDS







Gelatin Nanofiber

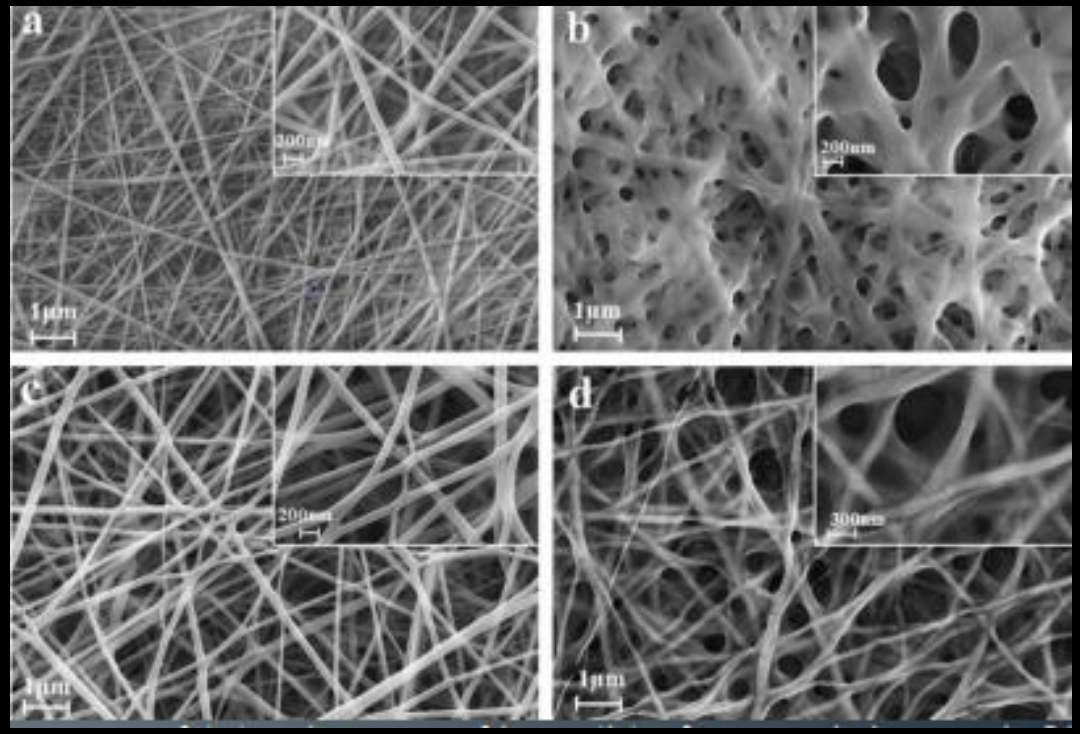
Electrospun

Crosslinking

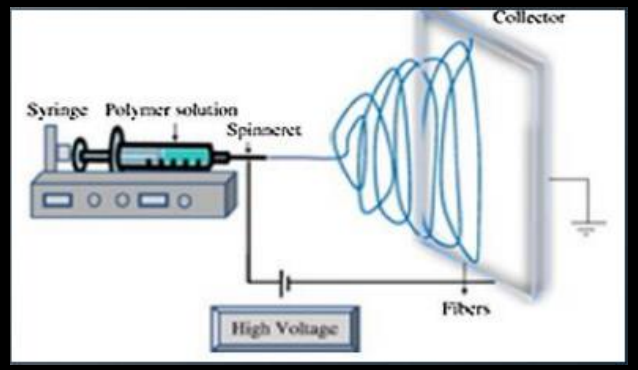
Long and uniform fibers with diameter range 50nm to 200nm

Stability in low pH

Slow to fast release: High to Low crosslinking



FESEM images of (a) gelatin nanofibers, (b) after crosslinking with GTA vapor for 6 min, (c) piperine loaded gelatin nanofibers, and (d) piperine loaded gelatin nanofibers after crosslinking with GTA vapor for 6 min

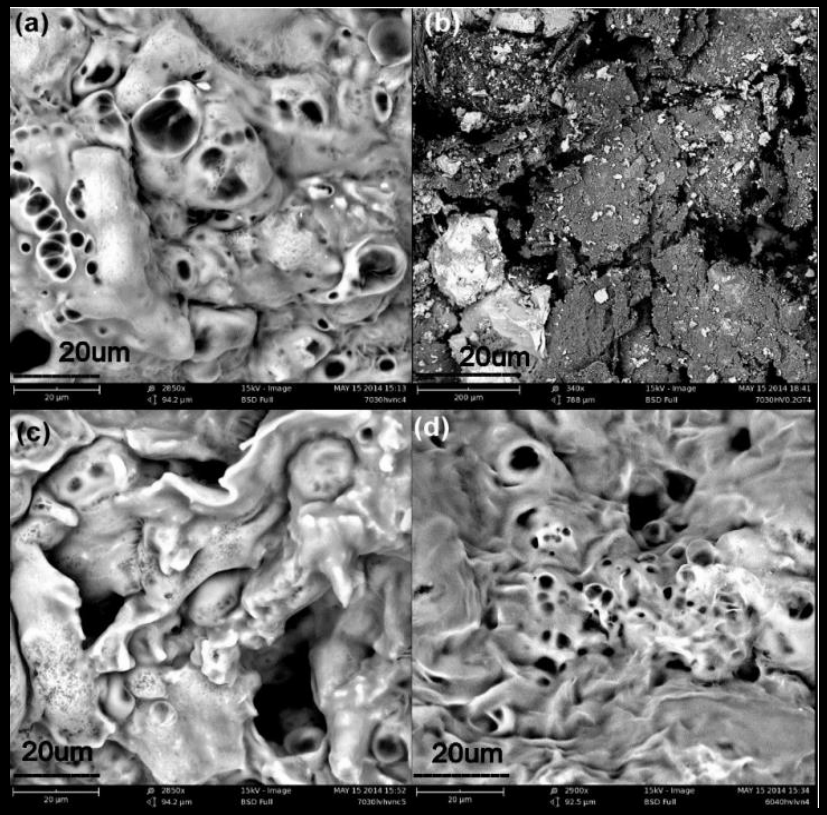


Anindita, Ph.D Student

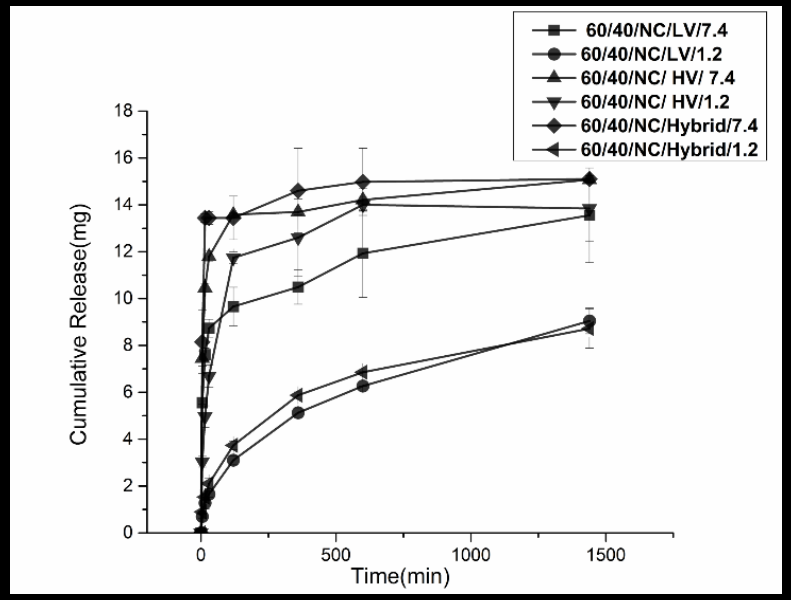
RSC Best Poster: Macro 15

***Biochemical Engineering Journal*, 2015, 105, 481**

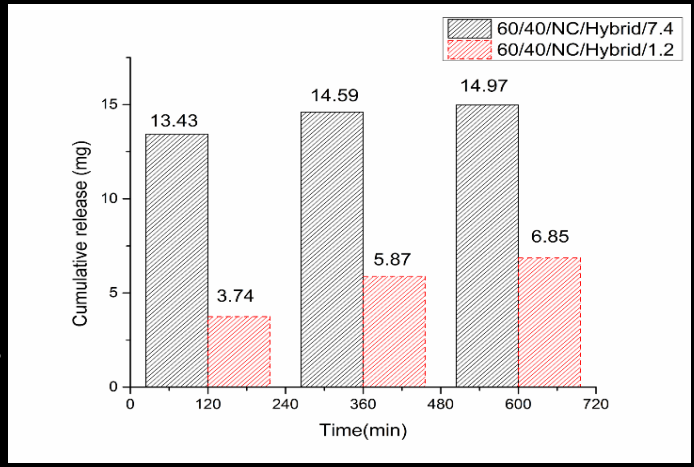
Controlled Drug Delivery: Hydrogel DDS



(a) 70/30 HV-NC (b) 70/30 HV 0.2% GTA
(c) 70/30 hybrid-NC (d) 60/40 hybrid-NC



Drug release study of 60/40 NC/LV/ HV and hybrid samples in pH 7.4 and pH 1.2

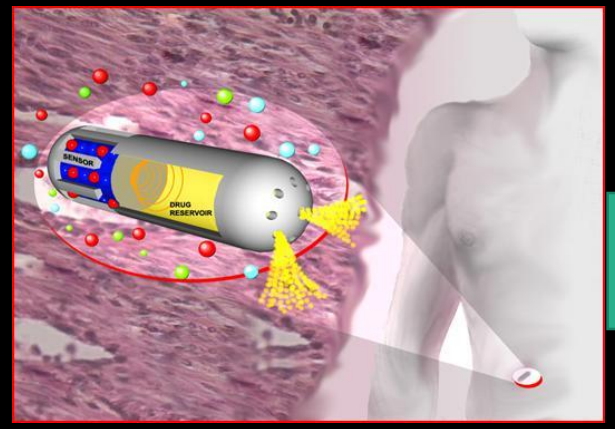


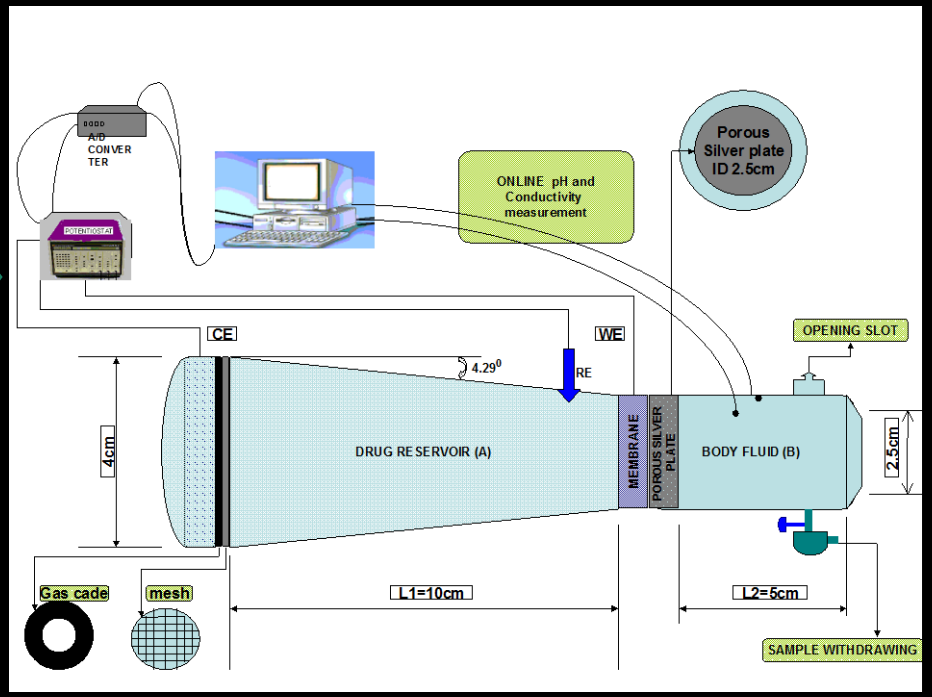
60/40/NC/hybrid pH 7.4 vs
60/40/NC/hybrid pH 1.2

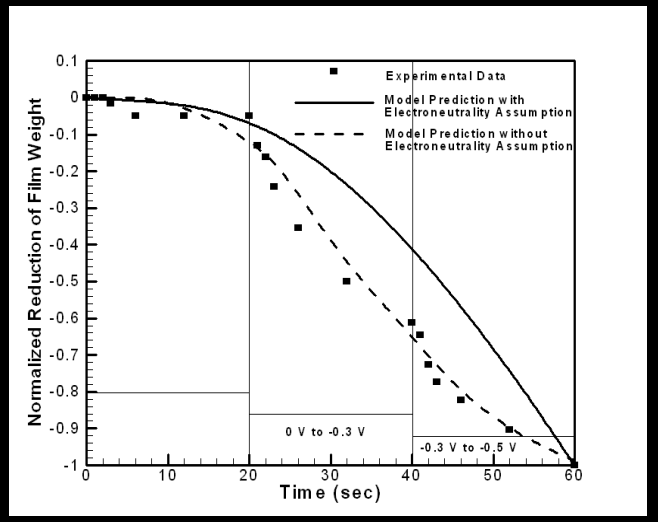
Utkarsh, Ph.D Student

Materials Letters, **2016, 164, 76–79**

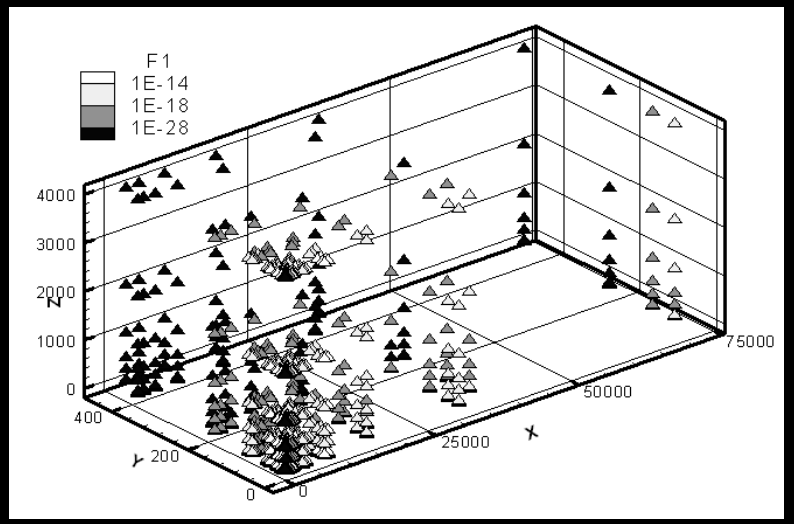
Conducting Polymer Based DDS

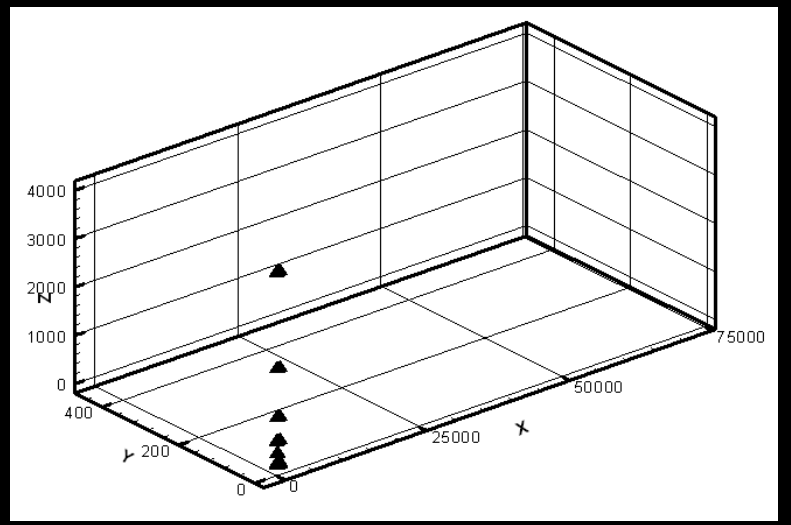






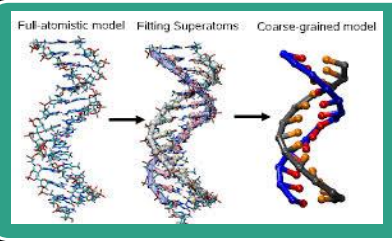
Comparison between flux data of Kontturi et. al. (1998) and simulation results for step-wise (voltage induced) release of salicylate molecules





- *CHEMPHYSCHEM, Vol. 11, Page 211-219*
- *Polymer Engineering & Science, Vol. 51, No, 10, Page 2001-2012*

Multi-scale Modeling: Coarse Graining



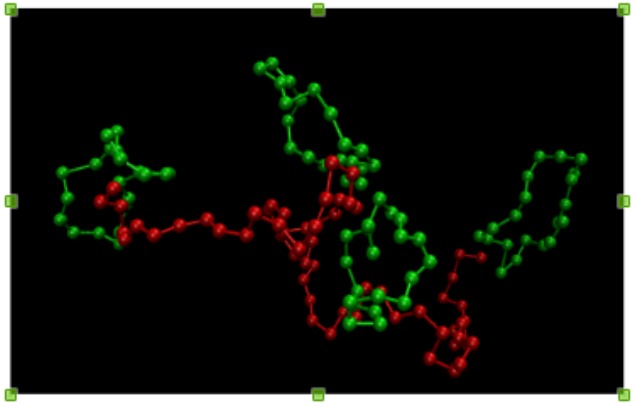
Coarse grained molecular dynamics

- Accessing length scale and time scale that classical MD can not reach
- Application to polymers under confinement

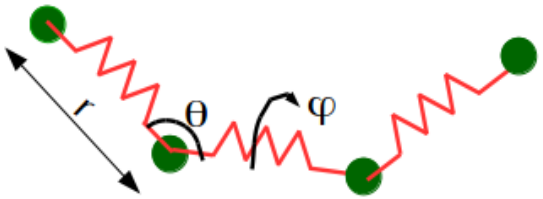
Pinaki, Ph.D Student

Work in Progress

Mapping from reference model to CG model



Reference model



CG model

Calculation of CG potential

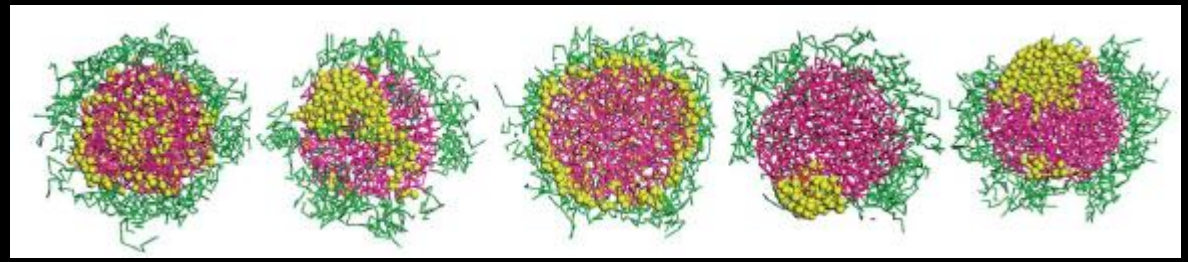
$$U^{CG} = \sum U^{CG}_{bonded} + \sum U^{CG}_{non-bonded}$$

Bonded potential

$$U^{CG}(r,T) = -k_B T \ln P^{CG}(r,T)$$

$$U^{CG}(\theta,T) = -k_B T \ln P^{CG}(\theta,T)$$

$$U^{CG}(\varphi,T) = -k_B T \ln P^{CG}(\varphi,T)$$



Guo et. al., Soft Matter, 2012

Multi-scale Modeling: Interfacial Tensions (DPD Application)

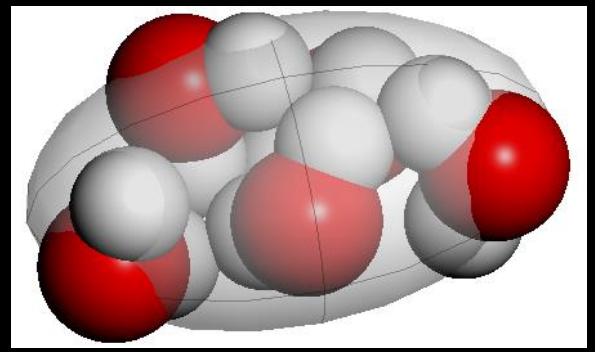
* Thermostat that Respects Hydrodynamics → ** Higher Length & Tine Scales → *** Relation with Stat. Mech. & Exp. Findings

TRANSFERABILITY

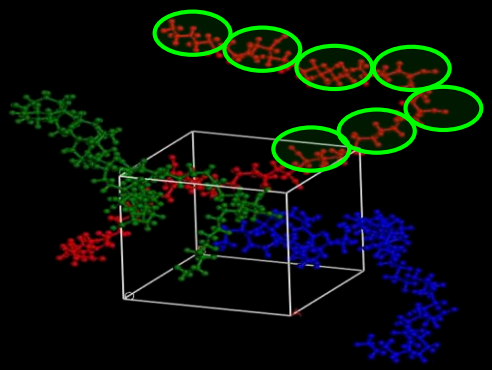
How to approximate the bead size and property in predictive fashion?

Can we reduce some assumption?

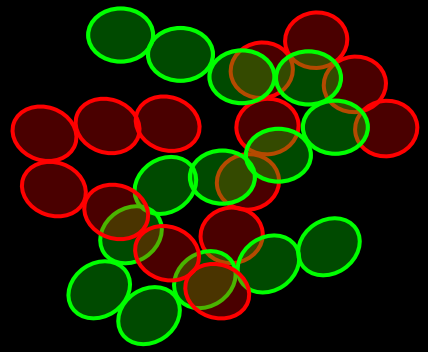
Is it better to use different experimental (or MD) findings for parametrization?



Molecular Dynamics



DPD

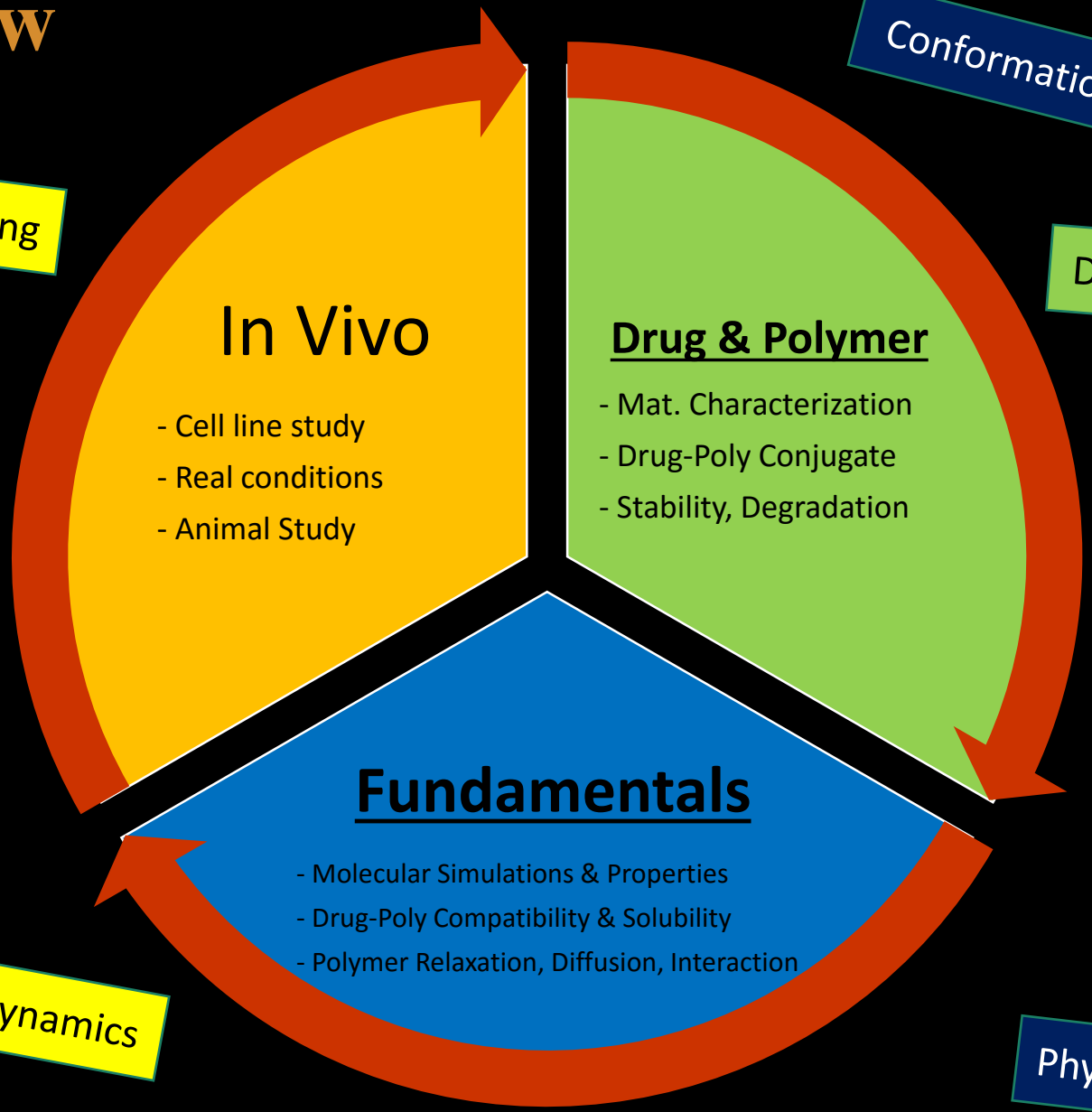


Himanshu

Rakesh

Chem. Phys. Lett. 600, 62 (2014)

Bird's Eye View



Surface Forces

Swelling

Solubility Parameter

Diffusion

Plasticizing/Screening

Solution Thermodynamics

Electrochemical Transport

Conformations

Miscibility

Dipole Interactions

Dispersion Force

Loop Interactions

Flory Parameter

Physical/ Chemical Cross Links

FOCUS OF THE DAY

5 Drug Delivery Vehicles

Natural Materials

Biodegradable Hydrogels/Nano-fibers/Thin Films/Conducting Polymers

Cargo Modifications

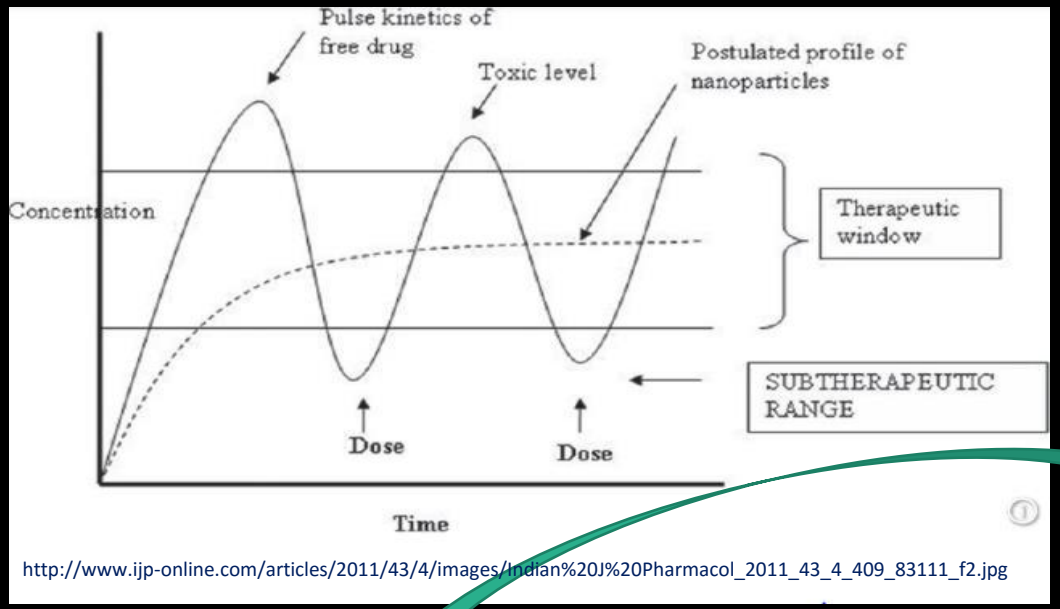
Control Release

Hydrogel

Utkarsh, Research Scholar

GYTI Award 2016

Drug Delivery Systems (DDS): Relevance & Encapsulation



http://www.ijp-online.com/articles/2011/43/4/images/Indian%20J%20Pharmacol_2011_43_4_409_83111_f2.jpg

Adv. Mater. **2015**, *27*, 65–72

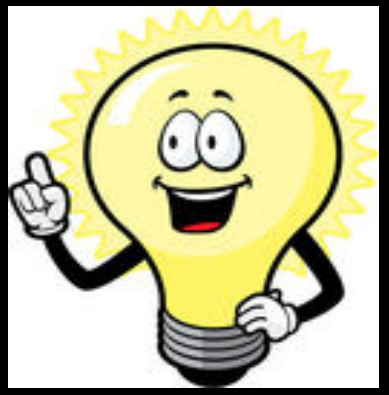
Despite their promise, many PEG hydrogels possess functional limitations that affect their prospects for clinical translation such as: (i) unfavorable toxicity and drug damage caused by the conditions/catalysts that facilitate the chemical reactions that lead to gelation,[18–20] (ii) impractical or uncontrollable gelation times,[21] (iii) the tendency for PEG networks to significantly swell upon equilibration and during degradation precluding their effective use in enclosed (fixed volume) in vivo environments,[22–24] (iv) lack of tunability of material degradation such that the material persists for periods well after the last amount of useful drug has been released exacerbating the foreign body response,[25,26] and (v) restricted spatiotemporal control of drug release resulting in high percentage of total drug-expelled over the initial hours of application.[12,17,21] It is important that these limitations be addressed if hydrogel materials are to be used as clinical drug delivery platforms.

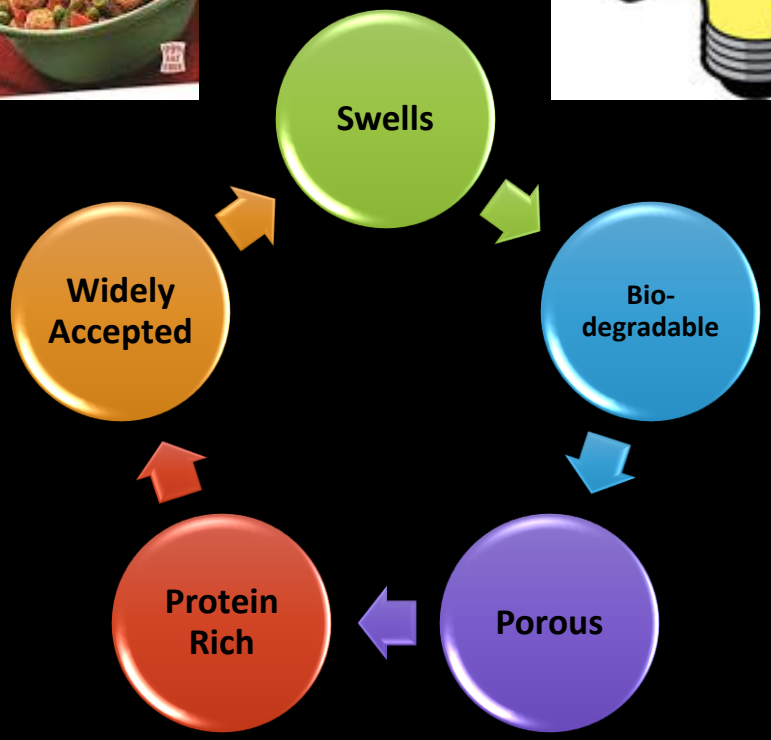
- **Cost of vehicle**
- **Natural/ widely accepted material**
- **High porosity & natural networks**
- **Stable under lower pH and enzymes**

Soya Nuggets – A Potential Carrier: Swelling Kinetics and Release of Hydrophobic Drugs

Characteristics of Polymeric Cargo

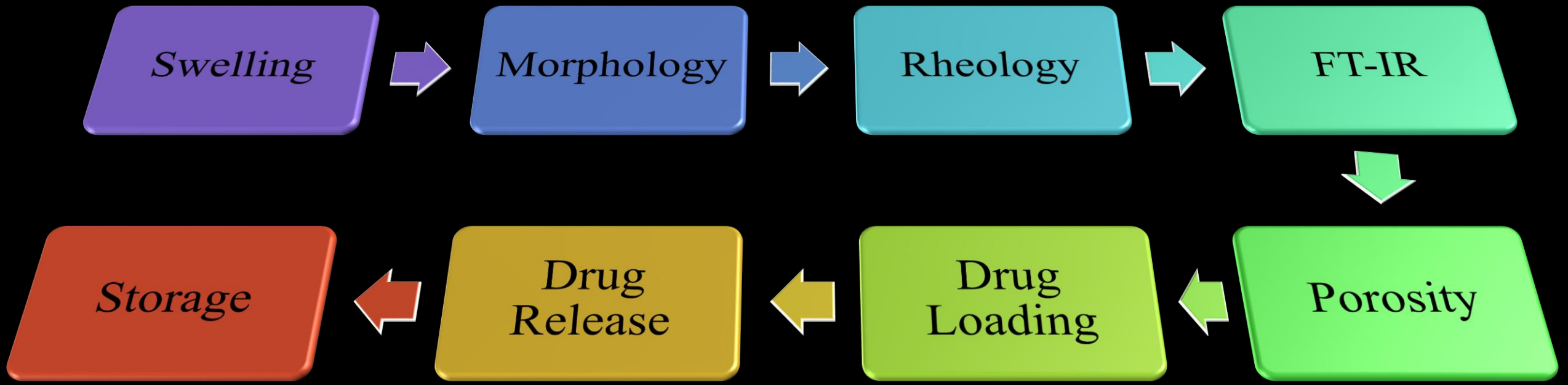
- Swelling
- Biodegradable
- Drug Encapsulation
- Control Release





Lets find out if it acts as a successful cargo

A STEP BY STEP APPROACH…



First Roadblock

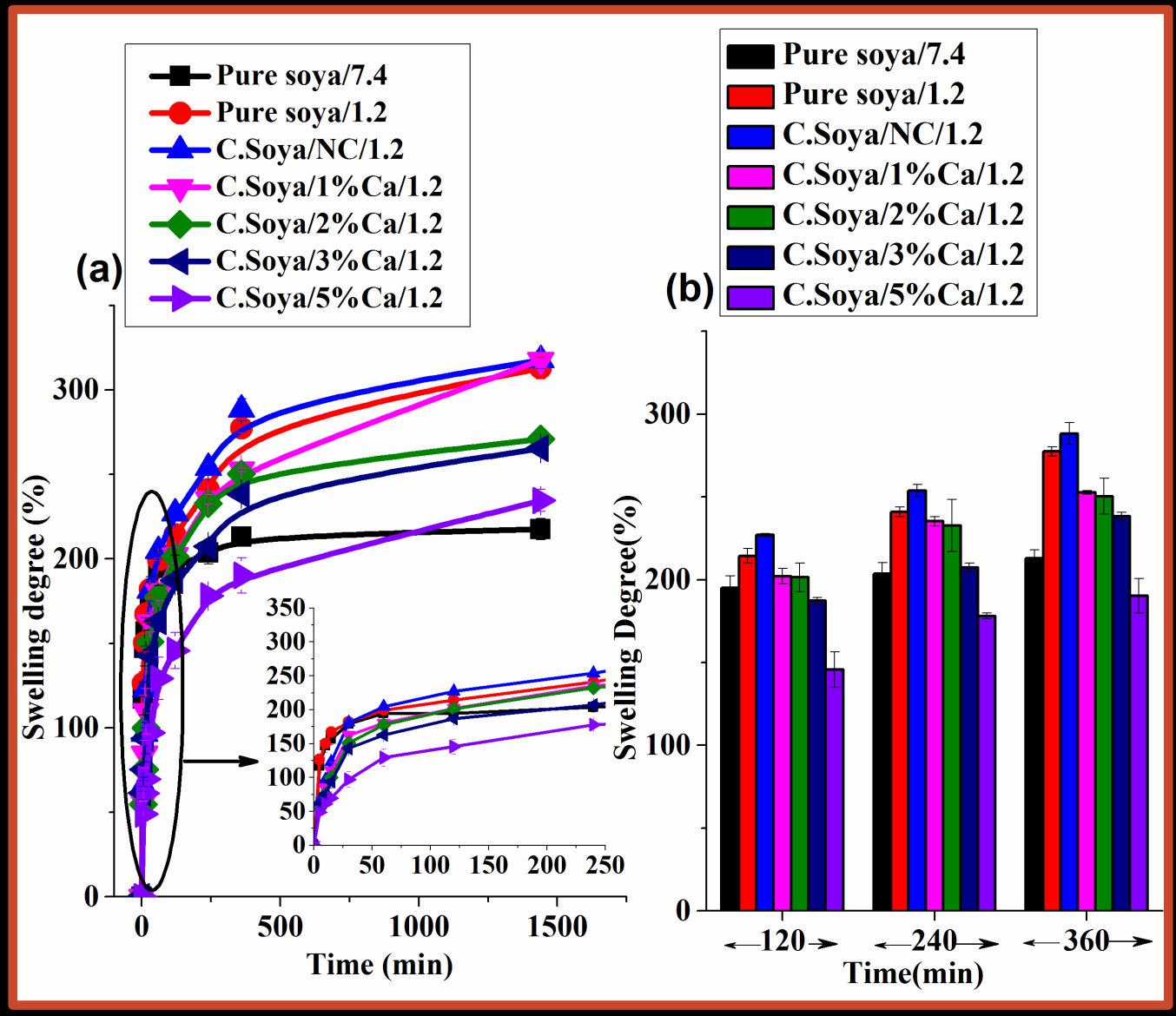
Swelling
- pH 1.2>> pH 7.4 (Not Favorable)

SA Coating
- Not Much Effect

$CaCl_2$ Crosslinking
- 1%,
- 2%,
- 3%,
- 5%

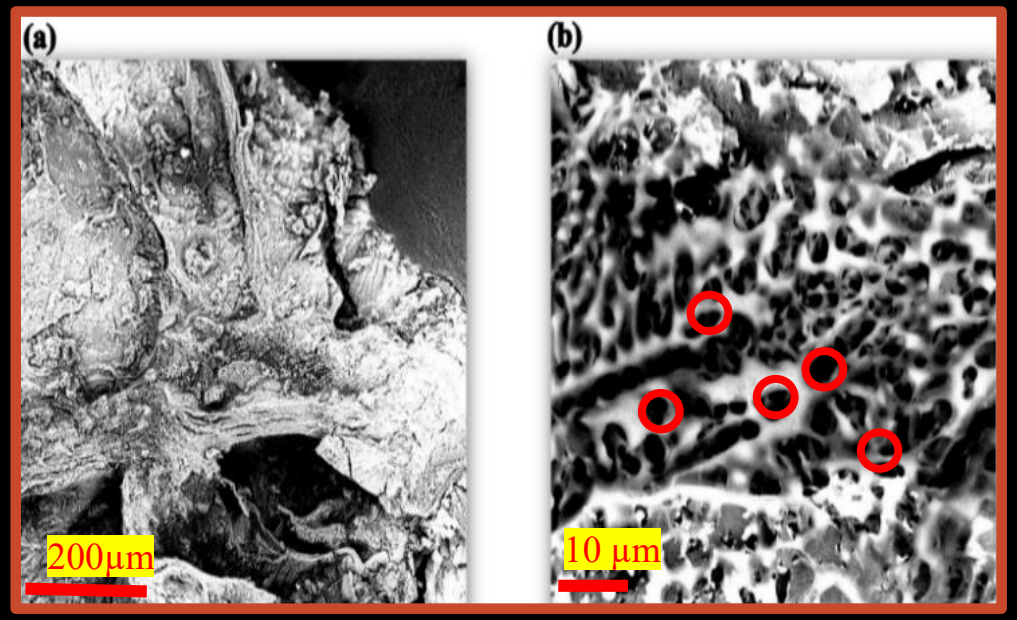
Why the nuggets swelled more in pH 1.2??
Was there any degradation observed??

$$SD\ \% = \frac{W_s - W_d}{W_d}\ X\ 100$$



Difficulty to Opportunity: Load the drug in low pH (i.e. 25/75 HCl/Ethanol)

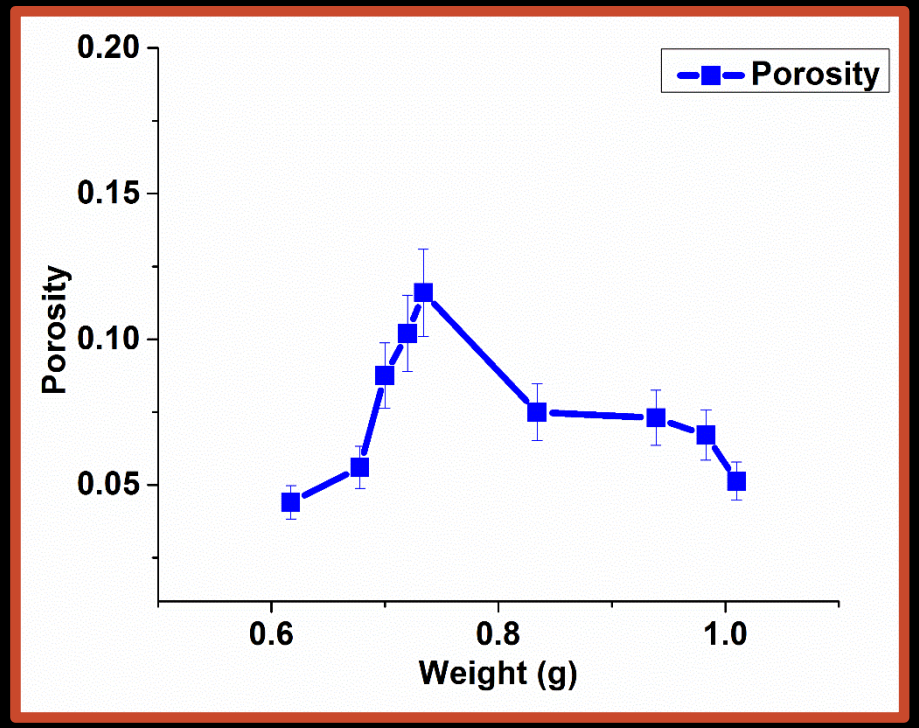
SEM

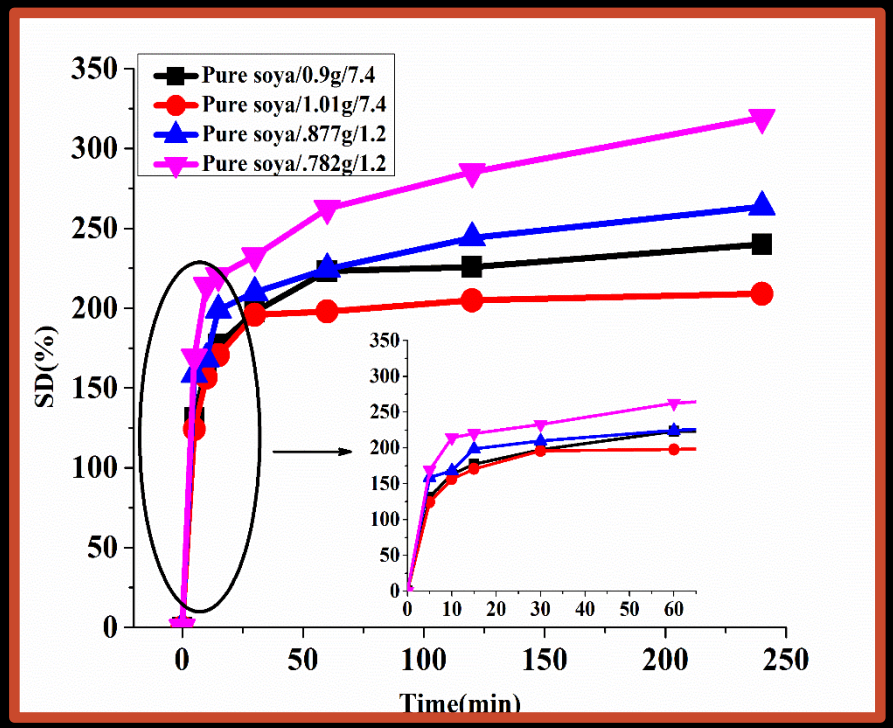


Pore Volume

Solvent Replacement

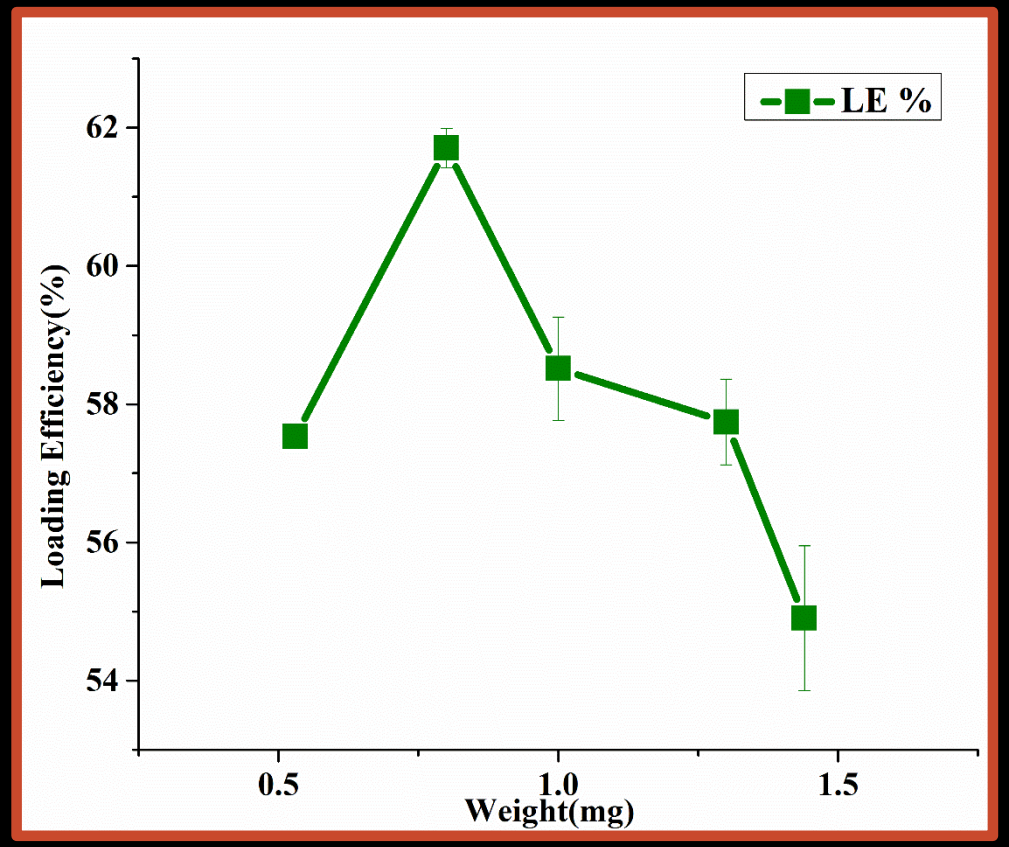
The Porosity first increased and then decreased

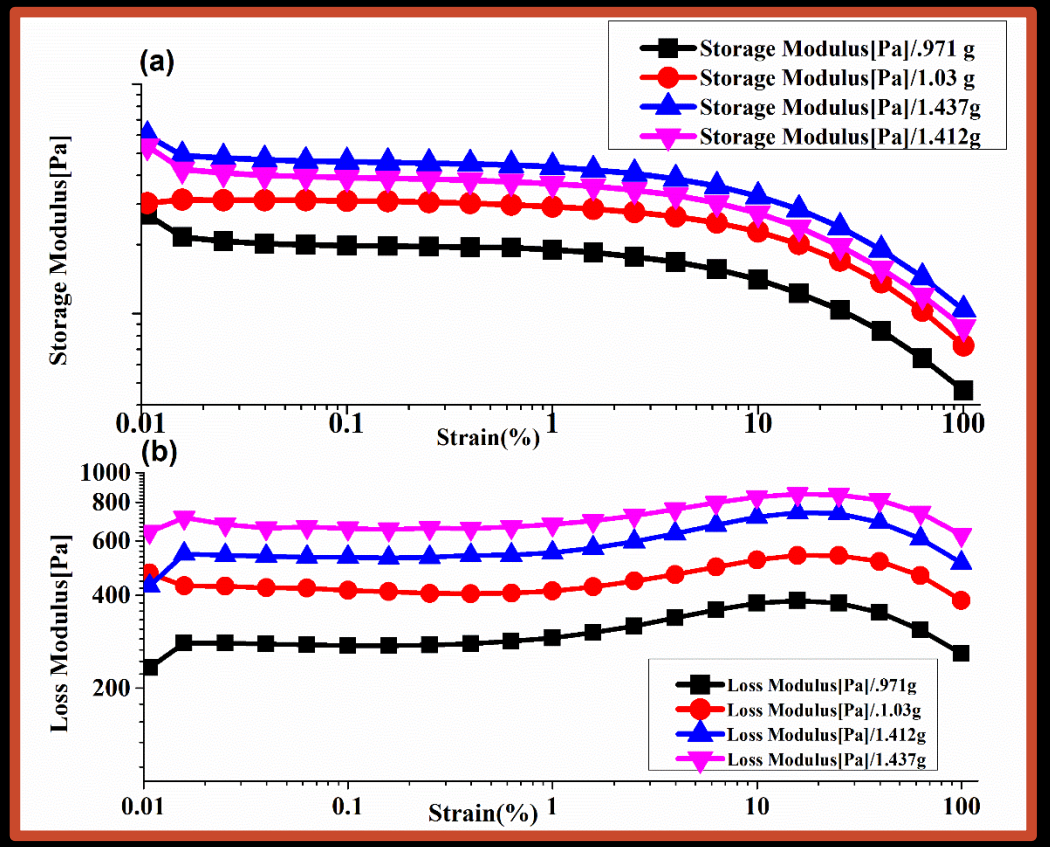




Higher the nugget weight
Lesser the SD

Second Roadblock





$$LE\ (\%) = \frac{Actual\ amount\ of\ drug\ inside\ the\ nugget}{Total\ amount\ of\ drug\ in\ the\ loading\ solution}\ X\ 100$$

- LE of Piperine increases and then decreases. Same trend was observed during porosity measurements.

Storage and Loss Modulus

- Higher nugget wt. higher storage & loss modulus.
- Higher modulus, lesser tendency to swell. That's why, higher weight nugget swelled less.

SWELLING KINETICS

Swelling of soya nuggets considered to be second order and the swelling rate at particular time interval expressed as

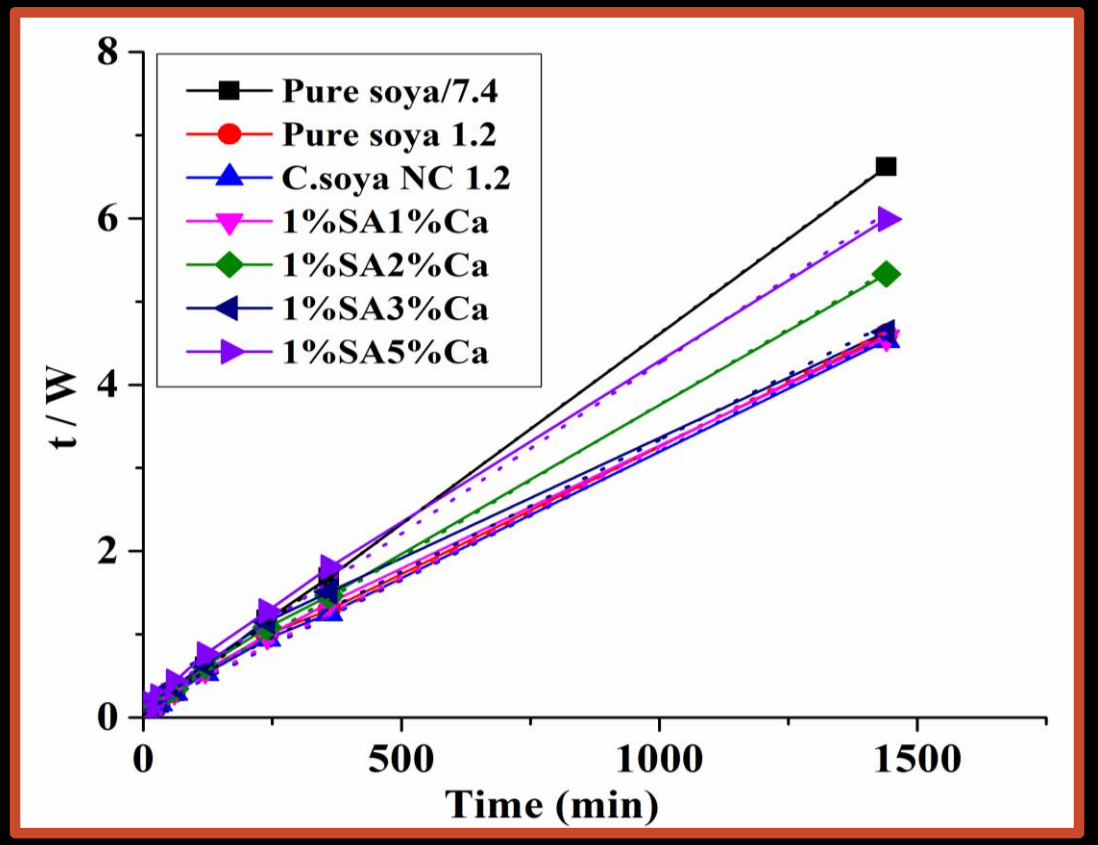
$$\frac{dW}{dt} = K\,(W_\infty - W)^2$$

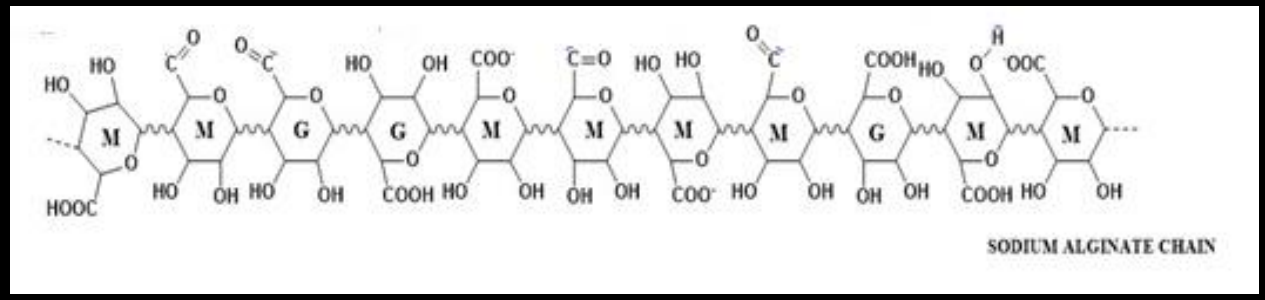
Integrating the above equation between t=0 and W=0,

$$W = \frac{K(W_\infty)^2 t}{1 + KW_\infty t}$$

Rearranging above equation:

$$\frac{t}{W} = \frac{1}{K(W_\infty)^2} + \frac{t}{W_\infty}$$



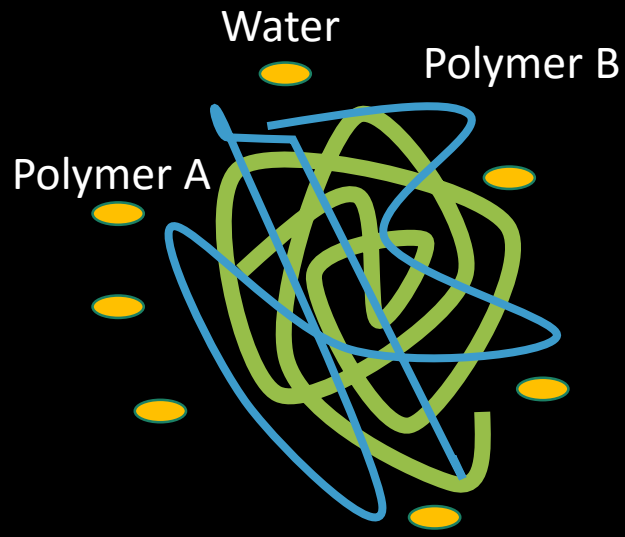


Power Law

$$\frac{M_t}{M_\infty} = K\,t^n$$

Diffusion Kinetics by Power Law

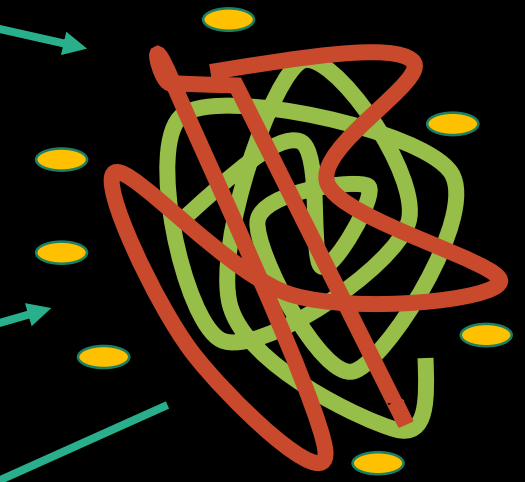
Sample/pH	Time (min)	n	Type of diffusion
Pure soya/7.4	60	0.18	Less Fickian
Pure soya/1.2	60	0.17	Less Fickian
1%SA/1%$CaCl_2$/1.2	240	0.326	Less Fickian
1%SA/2%$CaCl_2$/1.2	240	0.313	Less Fickian
1%SA/3%$CaCl_2$/1.2	240	0.28	Less Fickian
1%SA/5%$CaCl_2$/1.2	240	0.24	Less Fickian



Exponent n of the power law and drug release mechanism from polymeric controlled delivery systems of different geometry

Exponent, n			Drug release mechanism
Thin Film	Cylinder	Sphere	
0.5	0.45	0.43	Fickian diffusion
$0.5<n<1.0$	$0.45<n<0.89$	$0.43<n<0.85$	Anomalous transport
1.0	0.89	0.85	Case-II transport

Siepmann & Peppas, Advanced Drug Delivery Reviews 48 (2001) 139–157



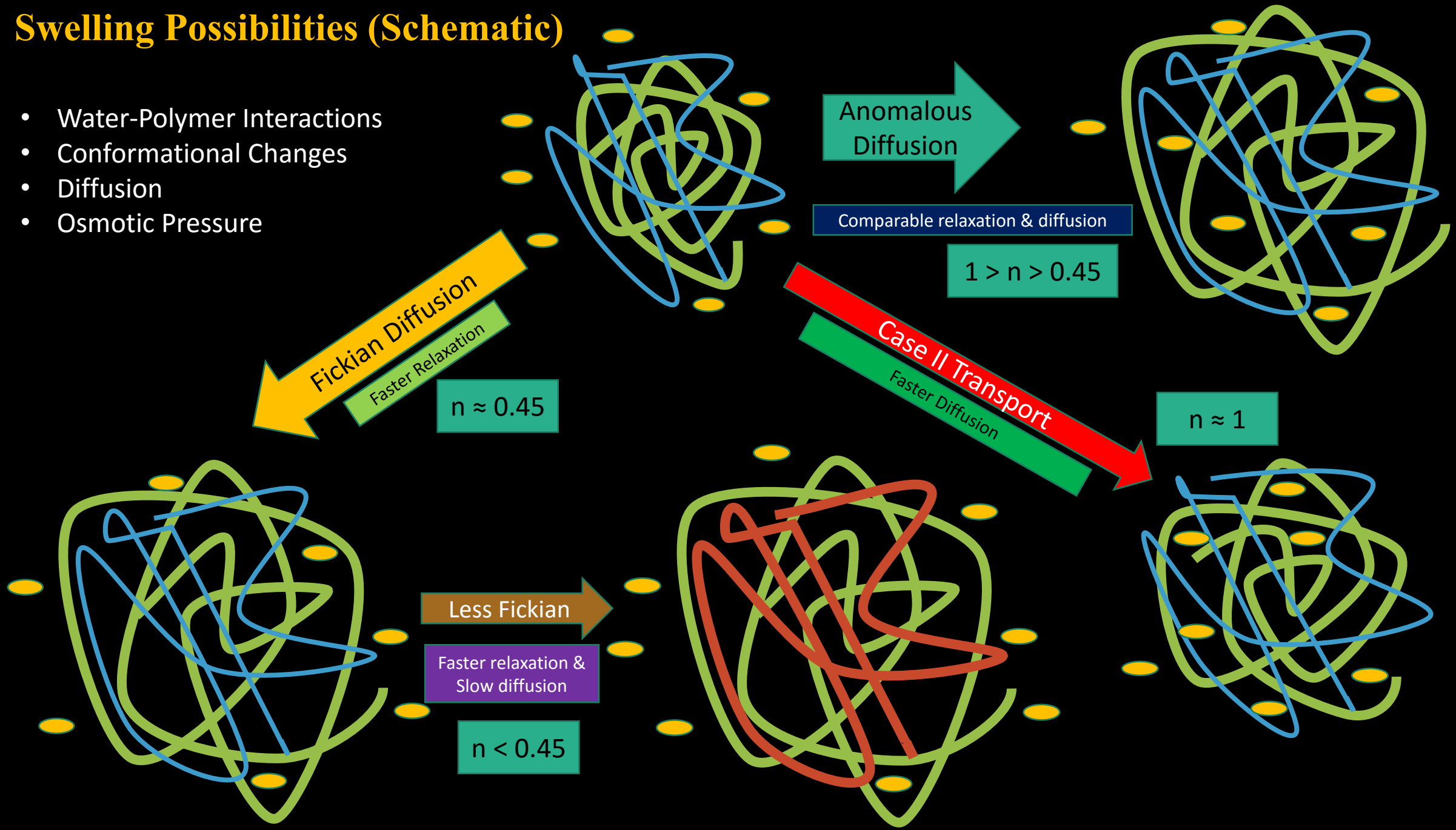
Polymer B: Hydrophobic

Wang J, Wu W and Lin Z. Journal of Applied Polymer Science, 2008; 109: 3018 – 3023

~20% Hydrophobic Content (Fibers and Fat etc.)

Swelling Possibilities (Schematic)

- Water-Polymer Interactions
- Conformational Changes
- Diffusion
- Osmotic Pressure



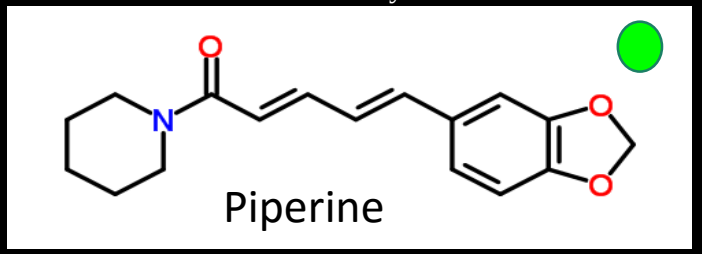
Release Possibilities in PBS 7.4 (Schematic)

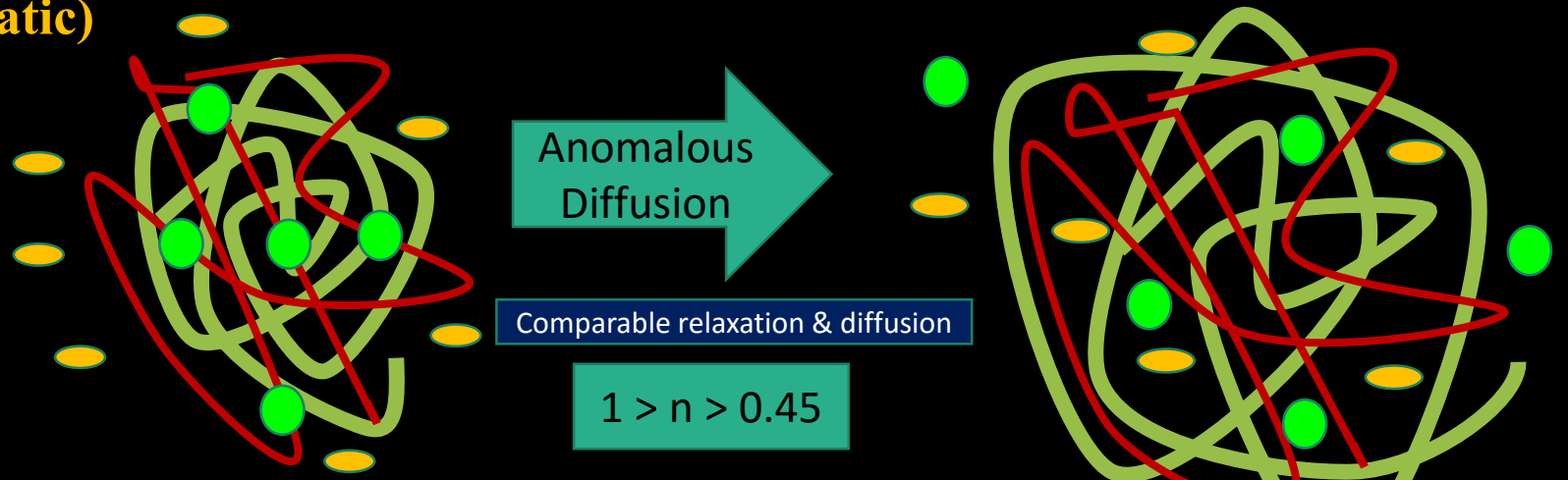
- Hydrophobic Drug (Piperine)

Release Kinetics by Power Law

Sample	n	Type of Diffusion
Pure soya/7.4	0.57	Anomalous Transport
1% SA NC 7.4	0.59	Anomalous Transport
1%SA 5%Ca 7.4	0.65	Anomalous Transport
1%SA 2.5%Ca 7.4	0.62	Anomalous Transport

For the applicability of power law for drug release, the cumulative release should be more than 60%. Only 4 cases satisfied the above condition.

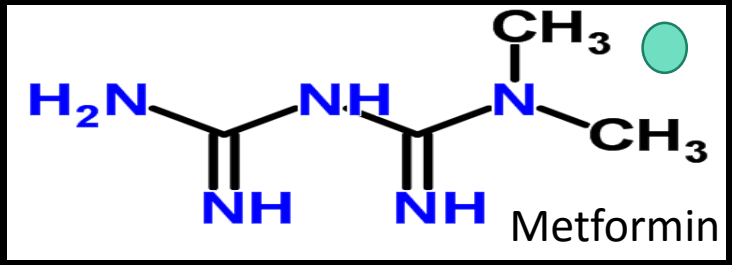


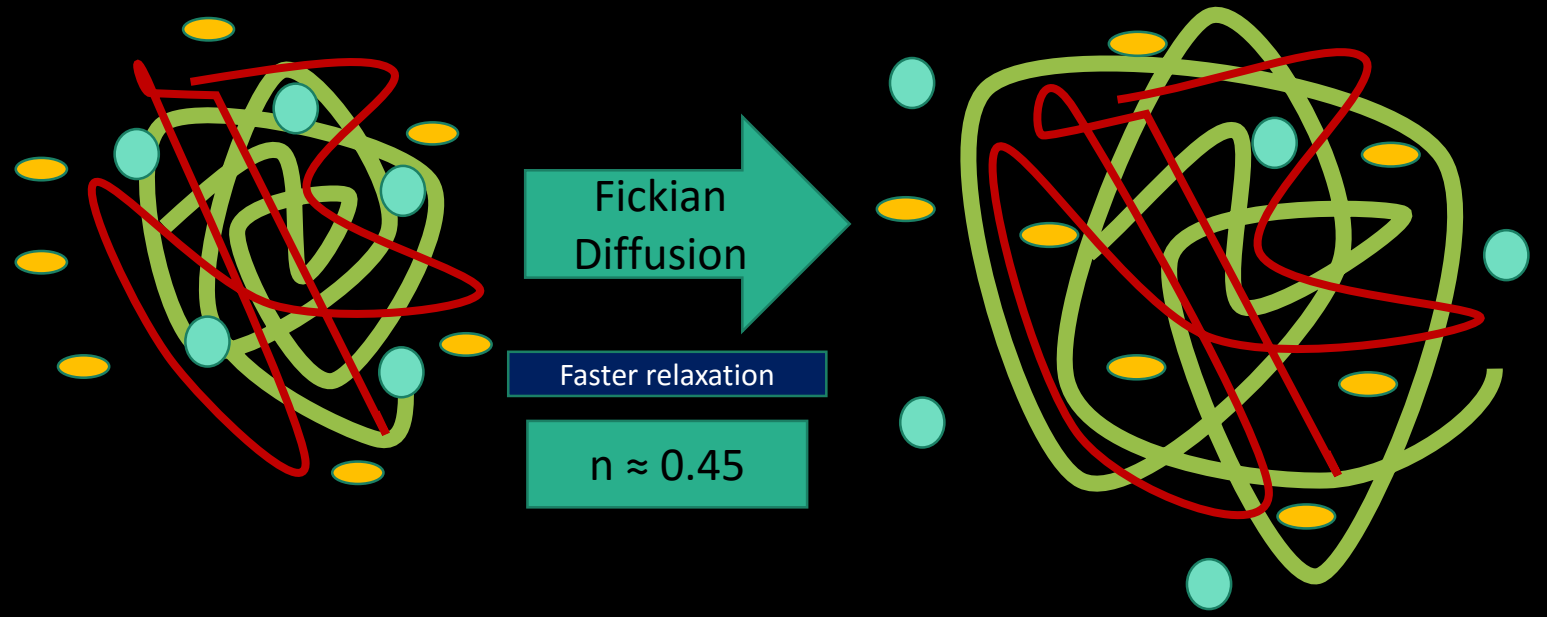


- Hydrophilic Drug (Metformin)

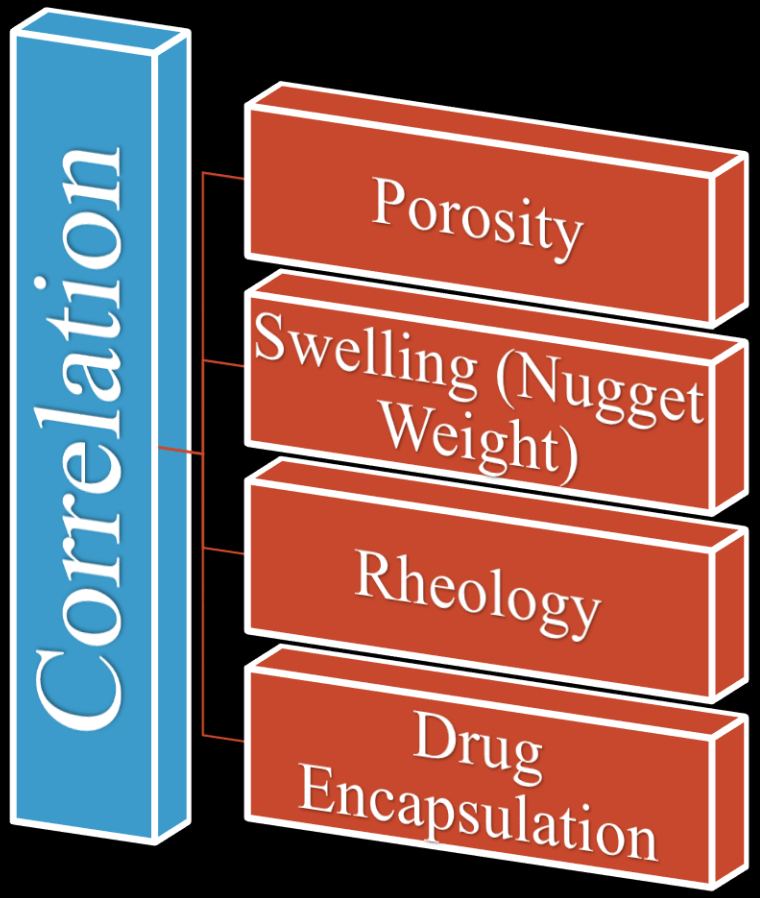
Release Kinetics by Power Law

Sample	n	Type of Diffusion
1% SA 5% Ca 7.4	0.46	Fickian





TILL NOW….



→ Increases and Then decreases with Nugget weight

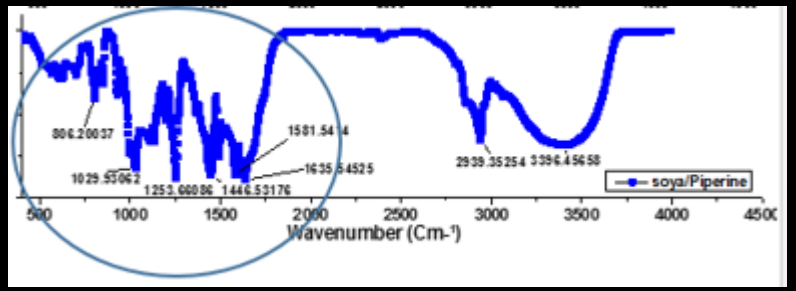
→ Lower weight nugget swells more

→ Higher the nugget weight higher the storage and loss modulus.
Higher the modulus, lesser the tendency to swell.

→ Drug Loading Increases then decreases with nugget weight

Lets Check the drug release now, but before that lets see the stability of the drug and chemical interactions

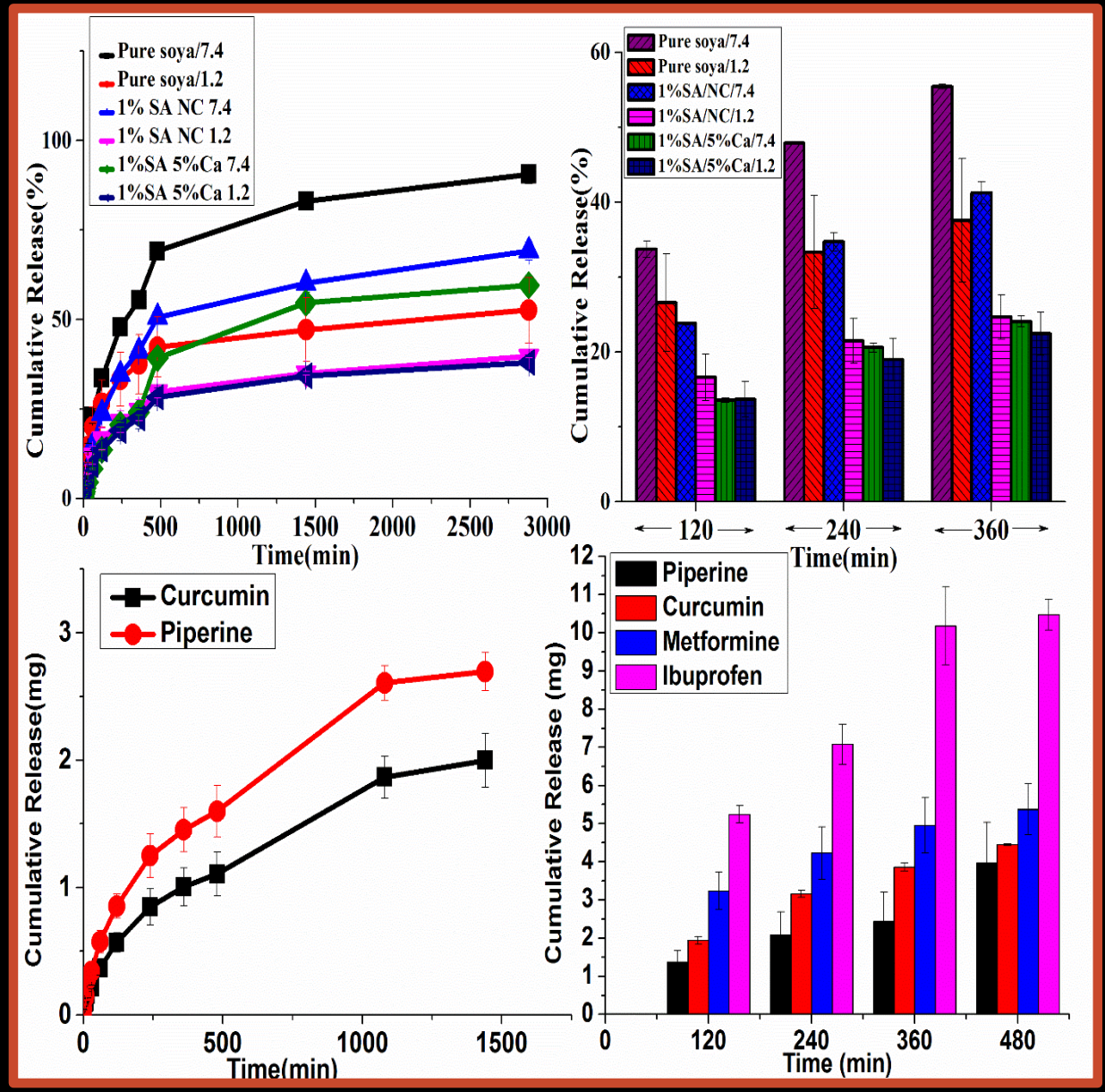
Soya+Piperine



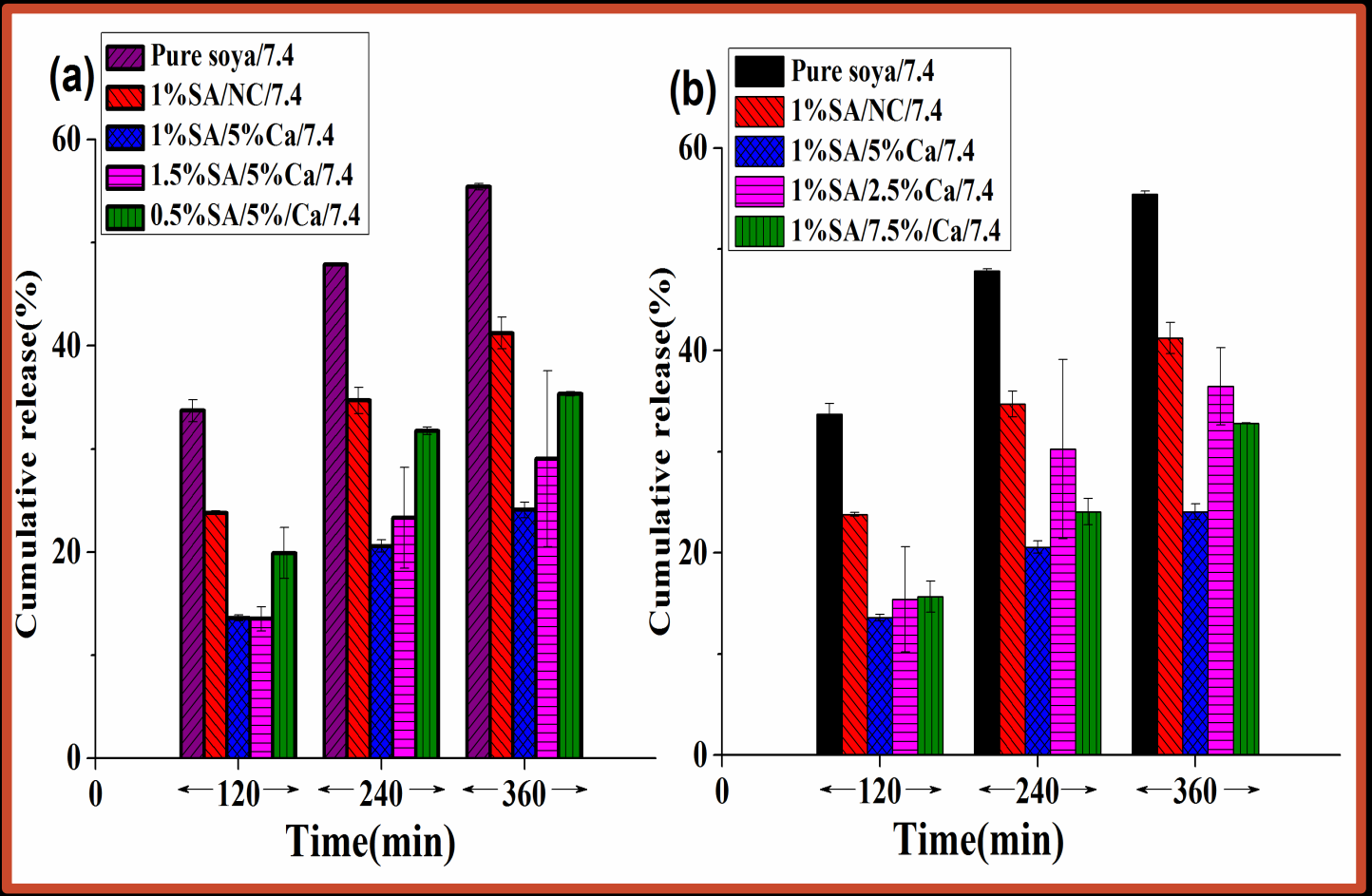
- **Drug stability also confirmed via FT-IR analysis**
- **The peaks in pure soya also confirm the presence of large number of protein groups**

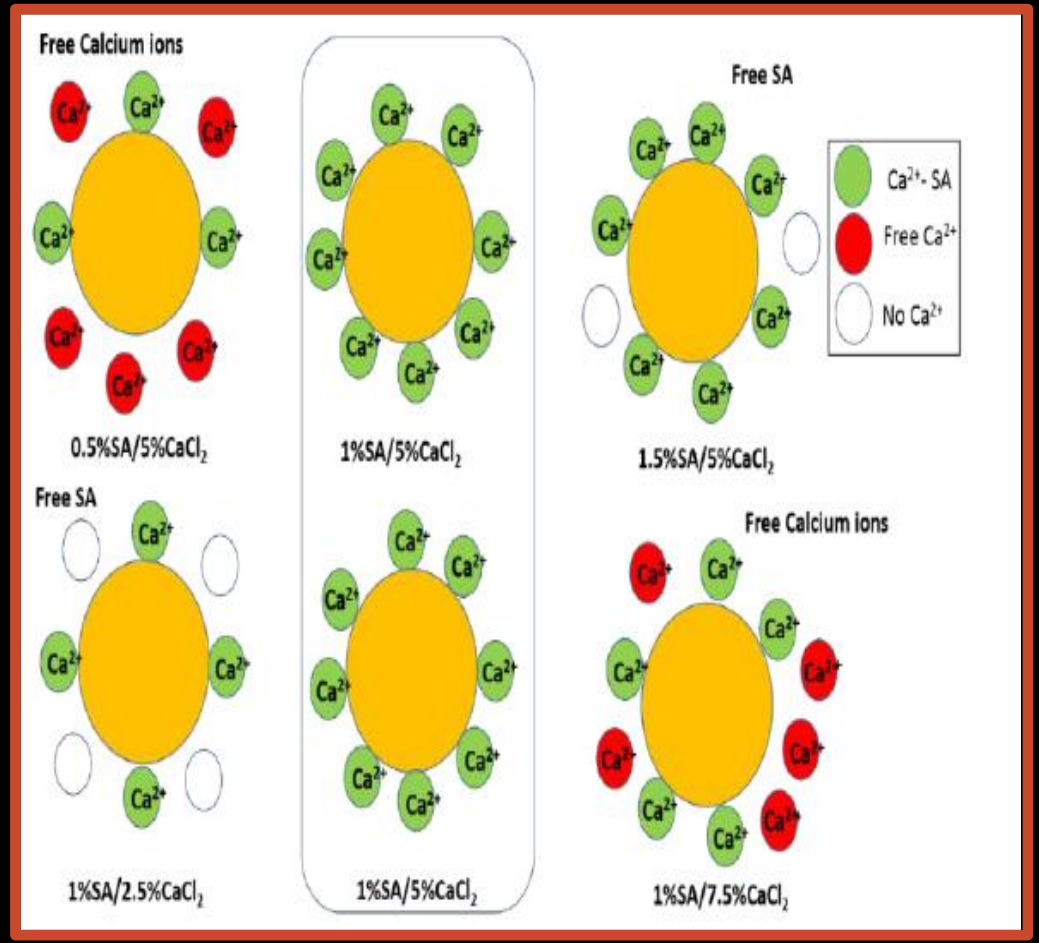
KEY FEATURES OF DRUG RELEASE

- Pure soya nuggets give a fast release in pH 7.4
- Soya nuggets in pH 1.2 were observed to give drug release of around 33% in the First 240 min.
- The soya nuggets were coated and cross-liked which led to a control over the drug release.
- 1% SA and 5 % CaCl2 cross-linked sample was observed to give a better release
- To further test the potential of nuggets, dual release of piperine and curcumin was tested.
- Piperine dominated the release as it enhances the bioavailability of curcumin.
- The nuggets were also tested with other drugs like metformin, ibuprofen.



Few parameters like concentration of SA coating and $CaCl_2$ were also varied and studied for drug release





1%SA/5% $CaCl_2$ offers a better control over the release of piperine

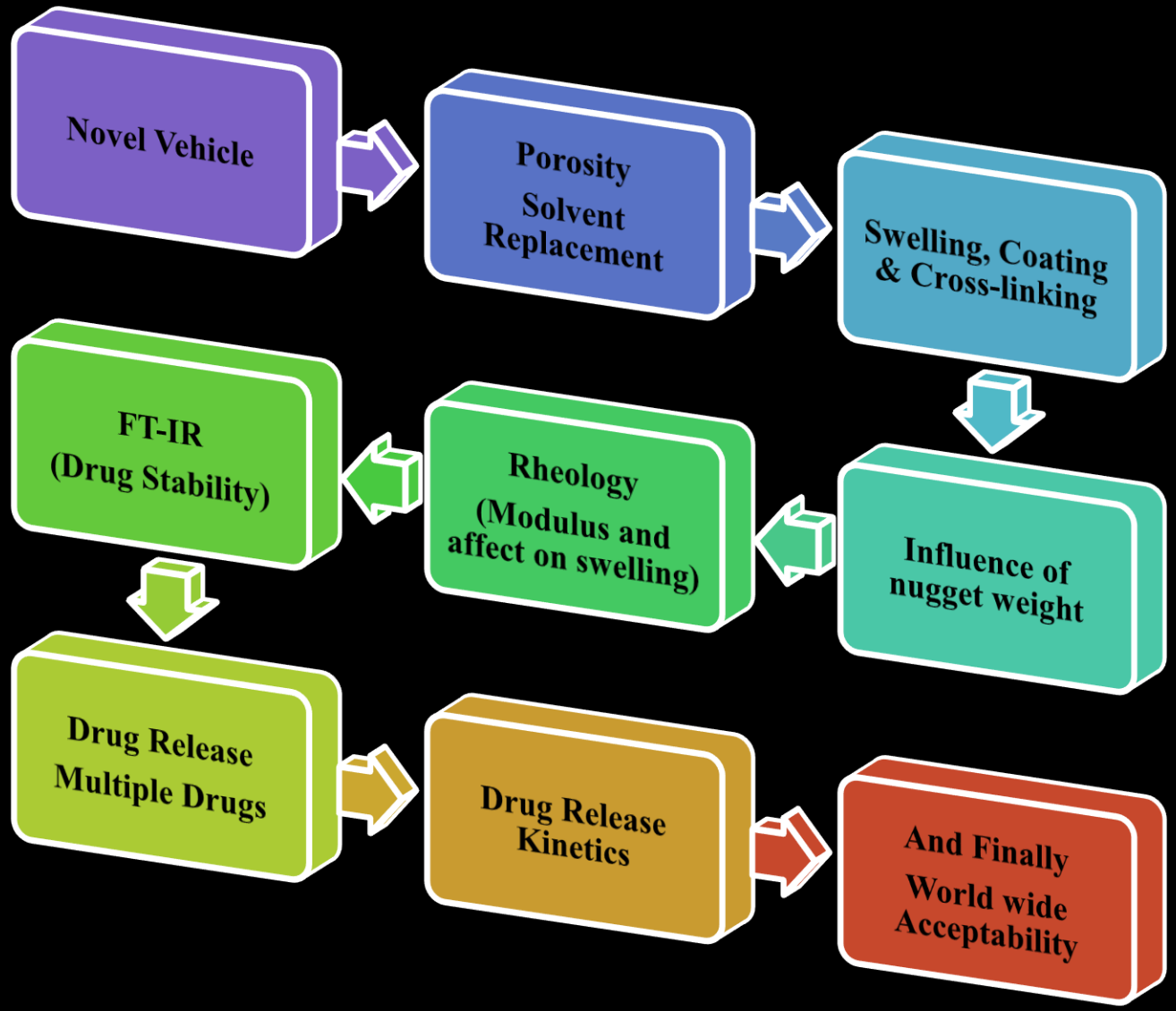
STORAGE OF DRUG LOADED NUGGETS

Drug Loaded Swelled Nuggets

Drug Loaded Swelled Nuggets wrapped and stored at 4°C

- Drug loaded nuggets were wrapped in aluminum foil & stored in refrigerator at 5-6° C for a month.
- It was observed that nuggets could retain their spongy nature when stored in cool conditions.
- Their spongy nature gives a rough idea about their storage possibility.

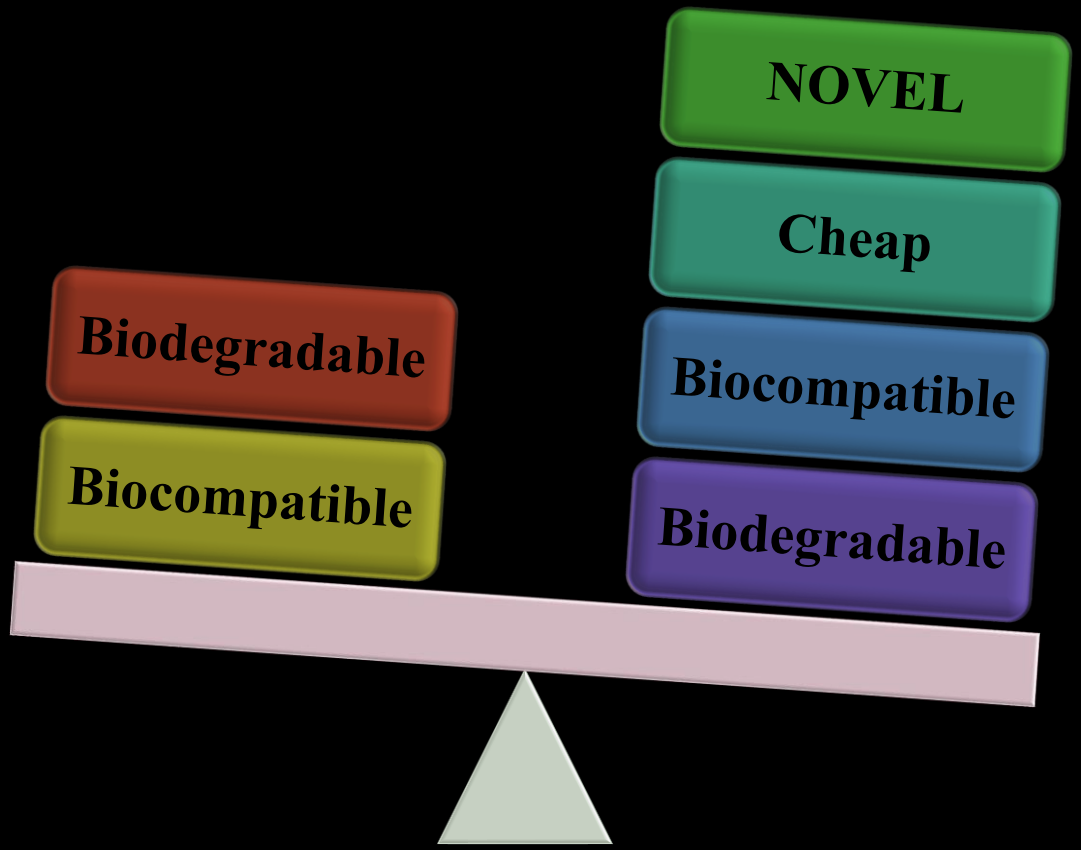
CONCLUSIONS



WHO WINS THE BATTLE????

Hydrogels

Soya Nuggets



WORK IN PROGRESS

Looking at any carrier from all possible aspects

- In-Vitro Cell line study: Dr. Lopa (IITH)
- Use of plasticizers for better drug distribution
- Increasing the loading efficiency of the vehicle
- Molecular modelling to predict the drug distribution: MD & DPD
- Incorporating widely used drugs

Scientific Contributions in Last Year (Drug Delivery)

- Utkarsh Bhutani, Anindita Laha, Kishalay Mitra, Saptarshi Majumdar, Sodium alginate and gelatin hydrogels: Viscosity effect on hydrophobic drug release, ***Materials Letters,*** **2016, 164, 76–79** ← Hydrogels
- Utkarsh Bhutani, Saptarshi Majumdar, Soya Nuggets – A Potential Carrier: Swelling Kinetics and Release of Hydrophobic Drugs, ***RSC Advances*****, 2015, 5, 92184-92188** ← Natural Materials
- Anindita Laha, Utkarsh Bhutani, Kishalay Mitra, Saptarshi Majumdar, Fast and Slow Release: Synthesis of Gelatin Casted-Film Based Drug Delivery System, ***Materials and Manufacturing Processes,*** **2016, 31, 223-230** ← Thin Films
- Anindita Laha, Shital Yadav, Saptarshi Majumdar, Chandra S. Sharma, In-vitro release study of hydrophobic drug using electrospun cross-linked gelatin nanofibers, ***Biochemical Engineering Journal***, **2015, 105, 481** ← Nano-Fibers

Expectation for Next Gen Oral DDS:

- High Drug Loading
- Cheap/ Min. Functionalization/ Natural Polymer
- Controlled Polymer Degradation (pH, Enzyme etc.)
- Less Toxic Chemicals (Min. Amount as well)
- Close to Zero-Order Release for ~48 Hours
- Complete Degradability of the Vehicle

Thank You

Use of Ultrasound to enhance antimicrobial efficacy

Dr Joey Shepherd

School of Clinical Dentistry, the University of Sheffield

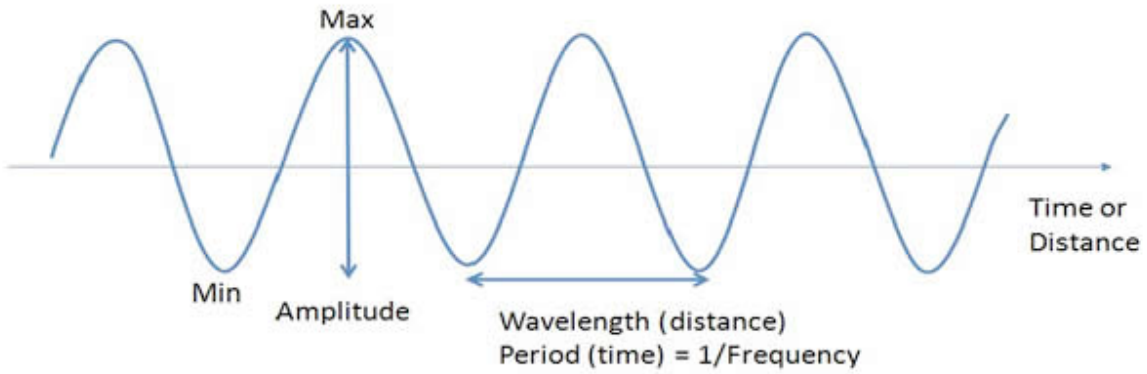
The University Of Sheffield.


HOTEL


skyscrapernews.com

Overview

- What is ultrasound (US)?
- US in antibacterial therapy
- Current projects
- US in opthalmology



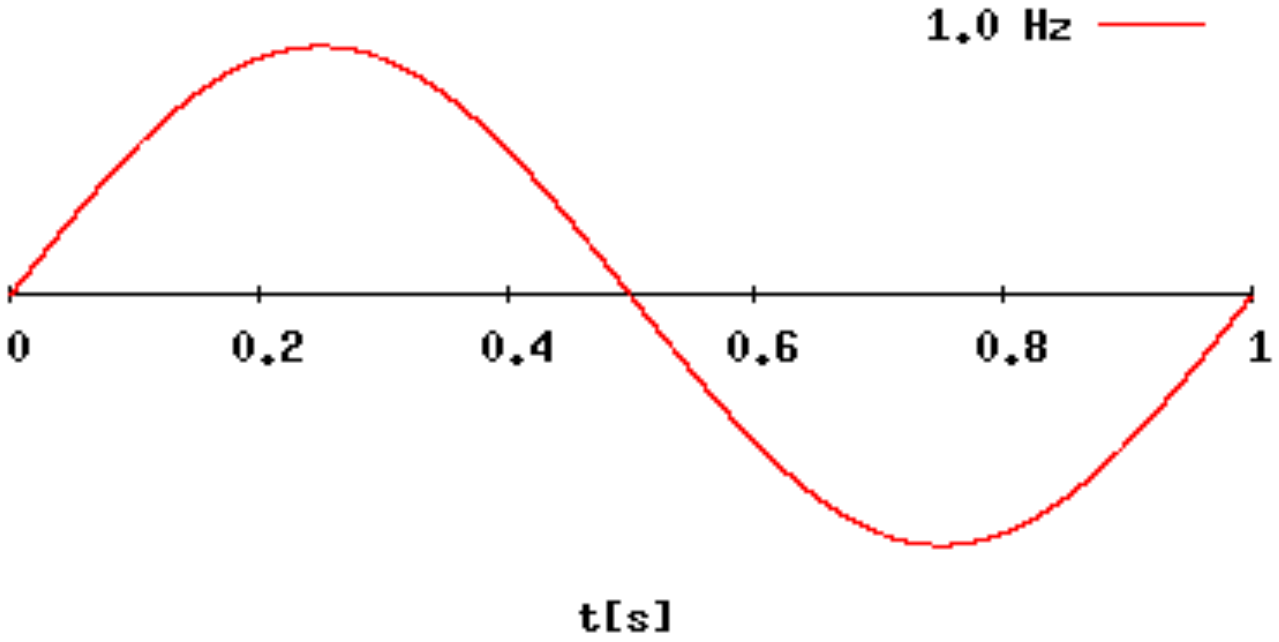
What is ultrasound?

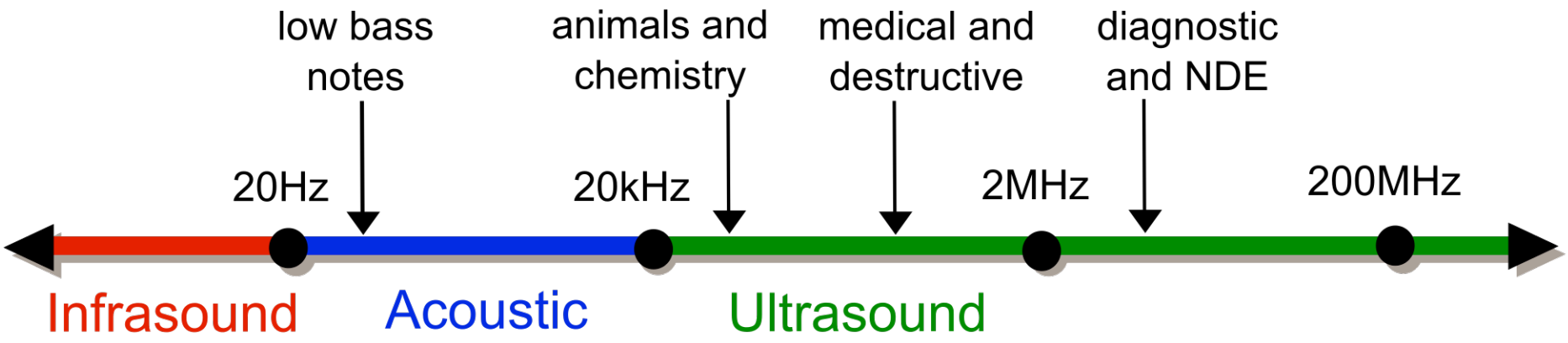
- Sound is a travelling longitudinal pressure wave
- Ultrasound = High frequency sound waves

- Frequency:
  - pulses per second
  - expressed in Hz (eg 10,000 cycles per second = 10 kHz).
  - Human hearing ~20 Hz-20 kHz
  - Ultrasound = >20kHz

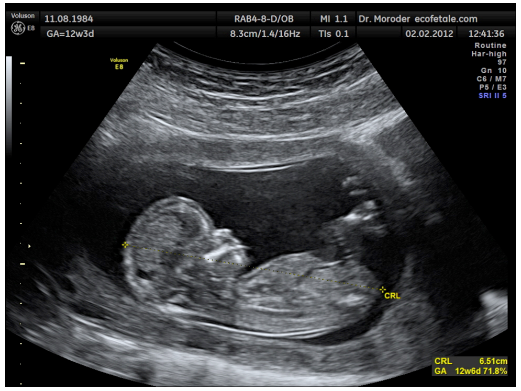
What's a Hz?

- 1Hz = 1 oscillation of the sound wave per second





- 2MHz - 10MHz medical diagnostic frequency range



- Low frequency US

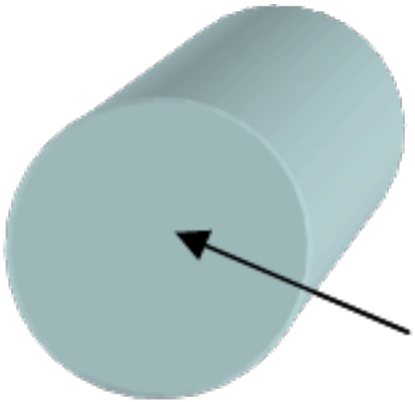
= 20kHz – 500kHz

Intensity

- Power: measured in watts (W);
  - amount of energy being produced by the transducer

- Intensity: strength of sound waves at treatment location

  - expressed in *watts per square centimeter (W/cm^2)*
  - Changing ultrasound head size affects power density (larger head results in lower density)



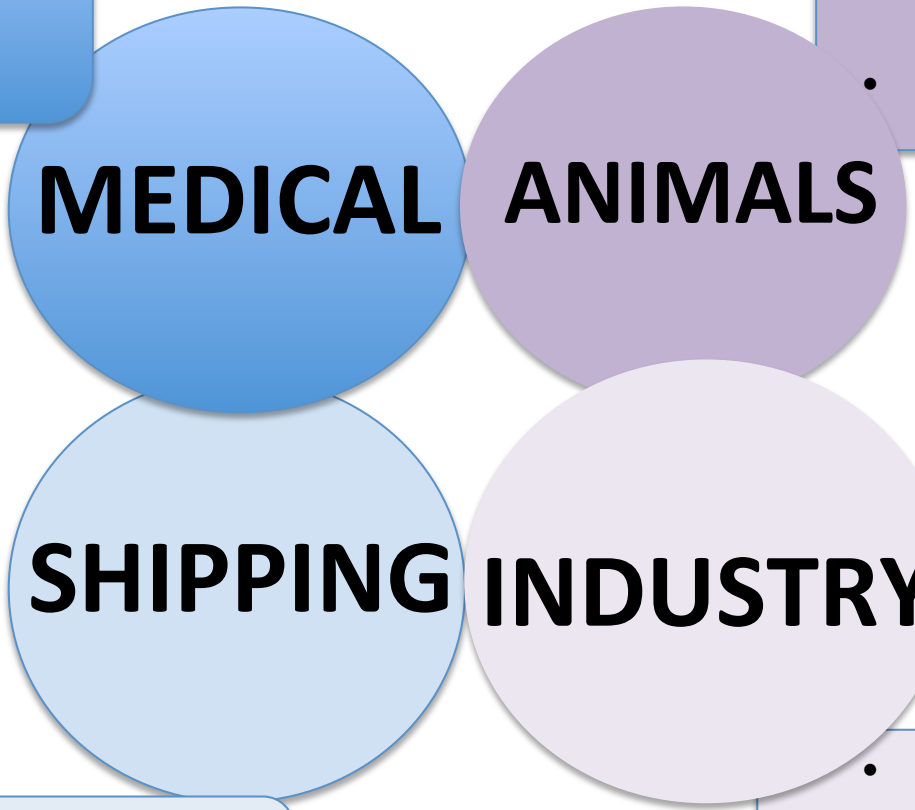
"Sound beam"

Cross-sectional area

$$intensity = \frac{power(mW)}{area(cm^2)}$$

Larger transducer head allows for lower intensity

Uses of US



- Imaging/diagnostics
- Bone fracture repair
- Descalers

- Echolocation: bats, dolphins
- Frog communication

- Echolocation of rocks/sandbars
- Echolocation of schools of fish

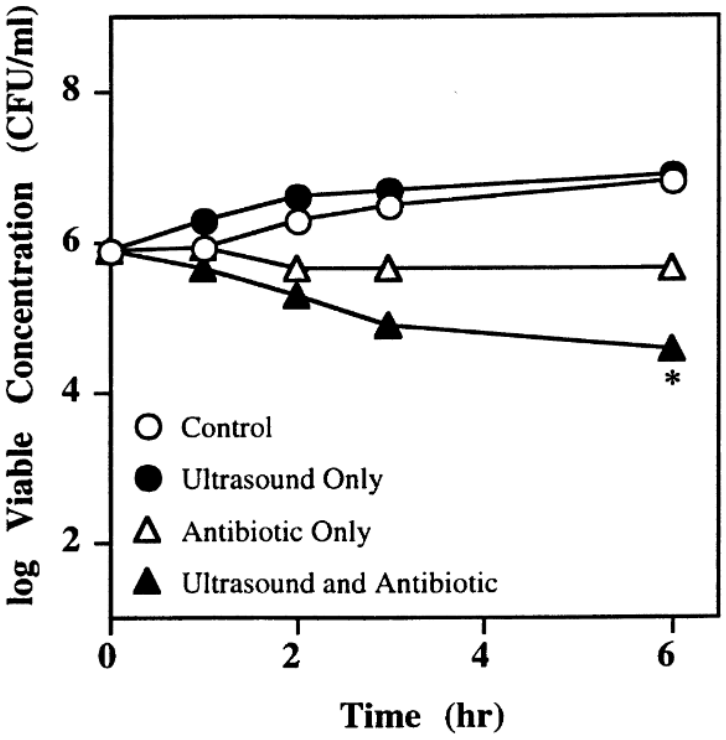
- Cleaning
- Mixing
- Cutting/engraving
- Food industry

US in antibacterial therapy

- Low frequency, high intensity US is used routinely for cleaning (ultrasonic baths)

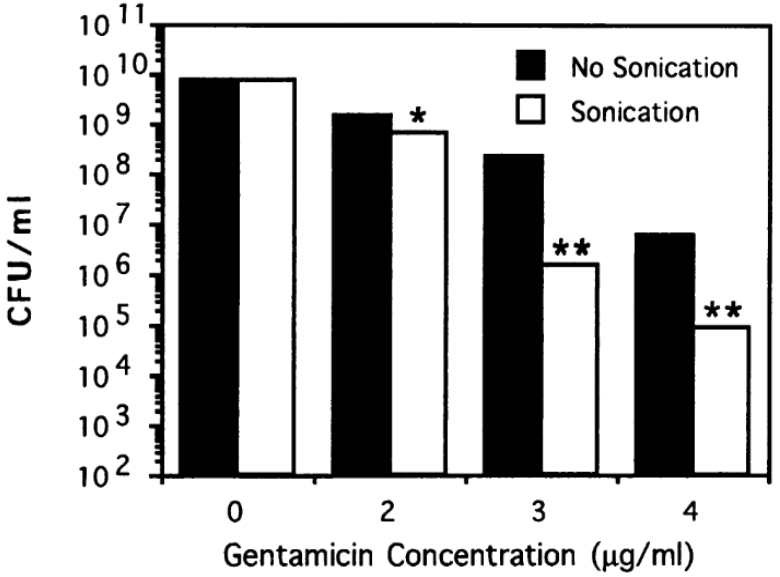
- Most research shows effectiveness of low frequency US when used in conjunction with antibiotics

***S.epidermidis*, +/- US +/- 75 µg/ml ampicillin**
70 KHz, 3W/cm²



Rediske *et al*
J.Gen.Appl.Microbiol 44:283

***P.aeruginosa*, +/- US +/- gentamicin**
67 KHz



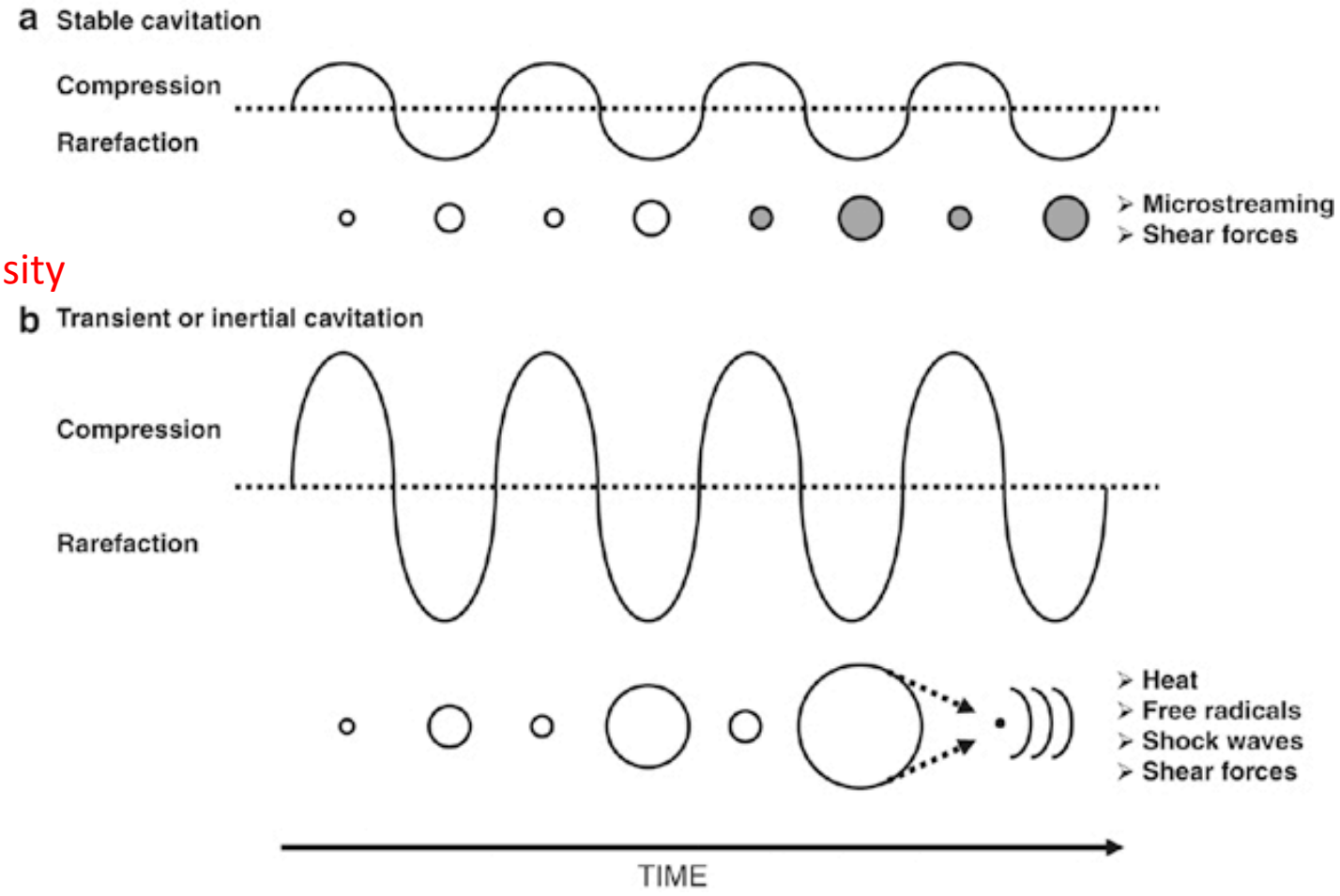
Pitt *et al*
Antimicrob.Agents.Chemother.
38:2577

Sonoporation

US creates pores in membranes allowing antibiotics to get into cells (‘sonoporation’)

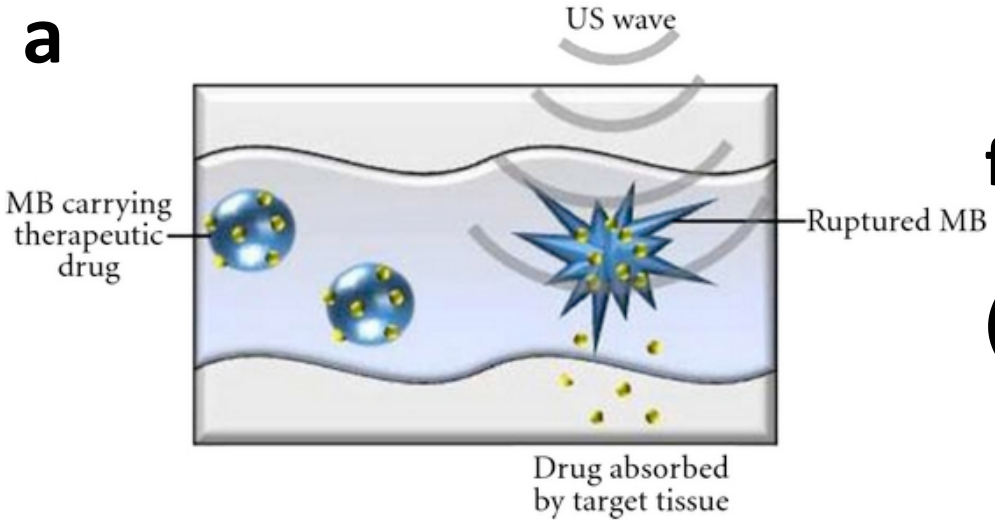
US-induced cavitation in liquids:

Low intensity



Low frequency in liquids

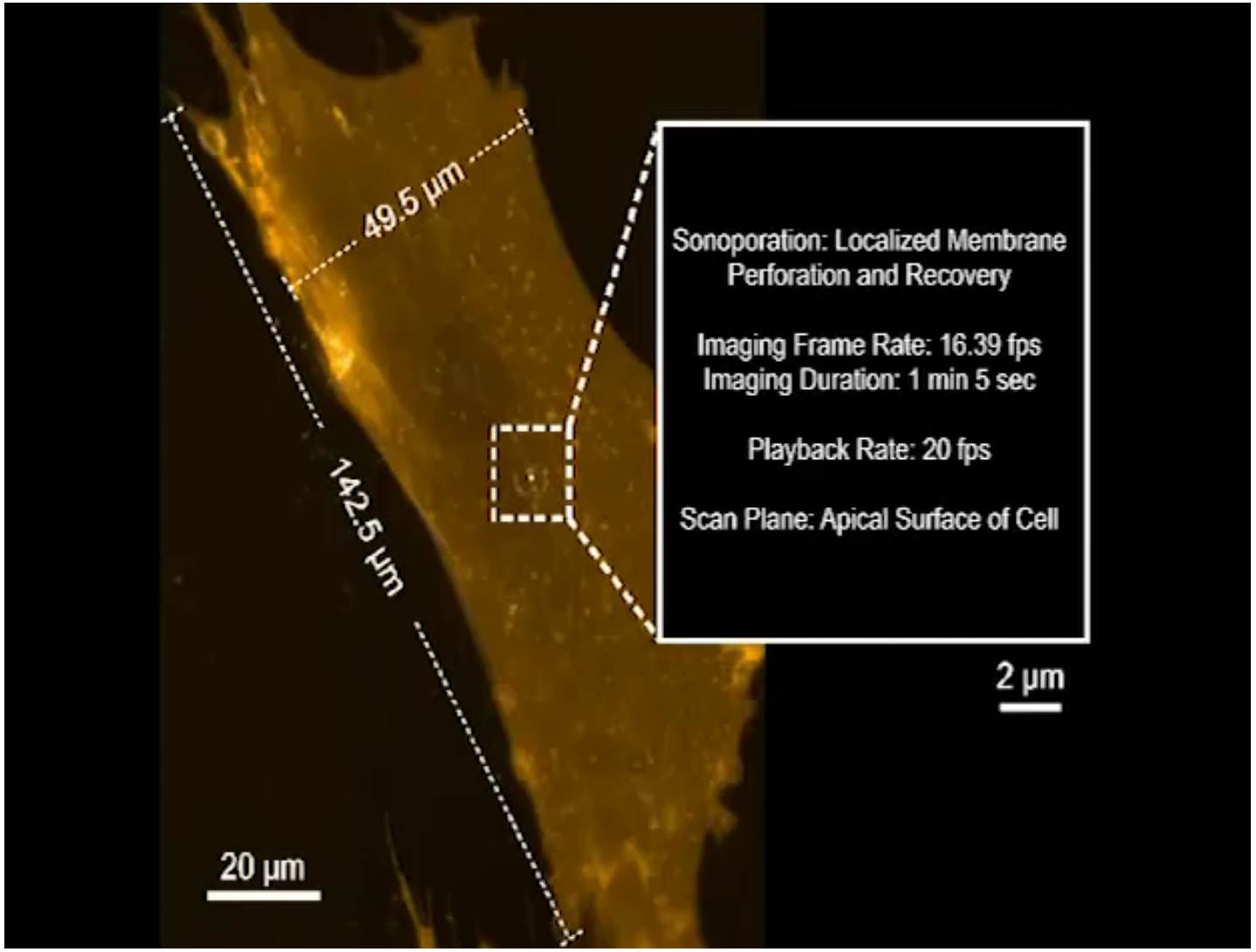
Sonoporation mechanisms for therapeutic delivery



(a) **Sonoporation for drug delivery**. Microbubbles with drug attached to the surface or enclosed within the particle travel in capillaries. Upon US exposure MBs rupture, releasing the drug contents. Drugs are absorbed by the target tissue.

(b) **Sonoporation for gene delivery**. plasmid-DNA-(pDNA-) containing MBs are passed through blood vessels adjacent to tumor cells, US waves rupture MB and release pDNA. Released pDNA penetrates into cells through their membranes by sonoporation.

Figueiredo & Esenaliev *J Drug Deliv* 2012



Real-time confocal imaging of membrane perforation and recovery in sonoporation induced by a single 2um microbubble adhered to an anchored fibroblast

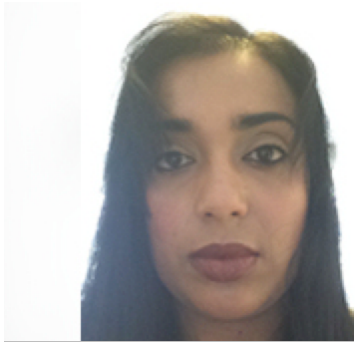
Hu et al *Ultrasound in Med & Biol* 39:2393 (2013)

Current studies

1) Low frequency ultrasound as antibacterial therapy

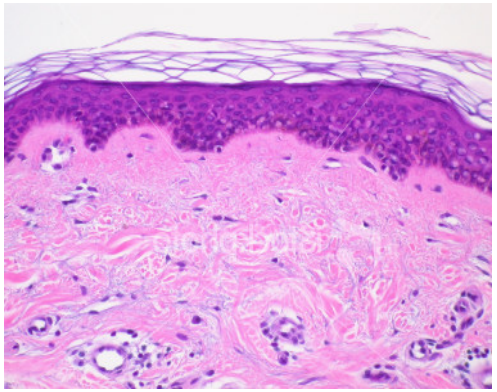
Alternative to antibiotics in chronic wound infections

Guma Beleid, PhD student
Dr Prachi Stafford
Dr Keith Miller

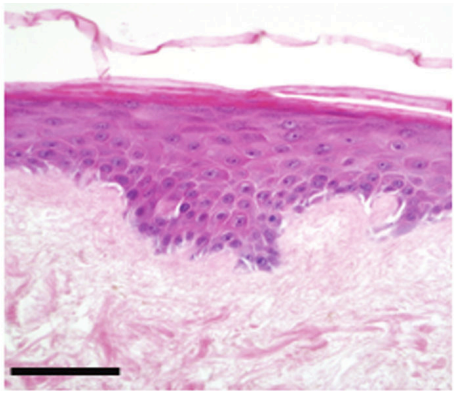


Sheffield Hallam University | Biomedical Research Centre

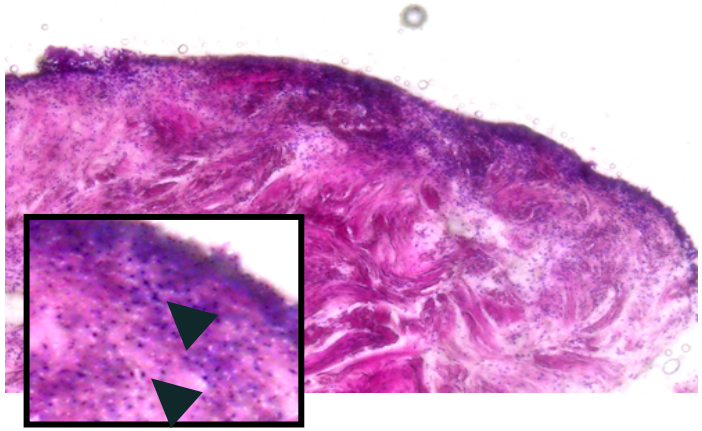
Infected TE skin model



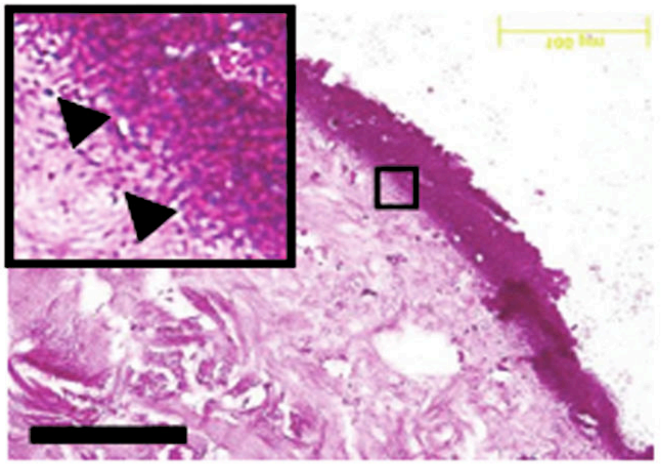
Human skin H&E



TE skin H&E



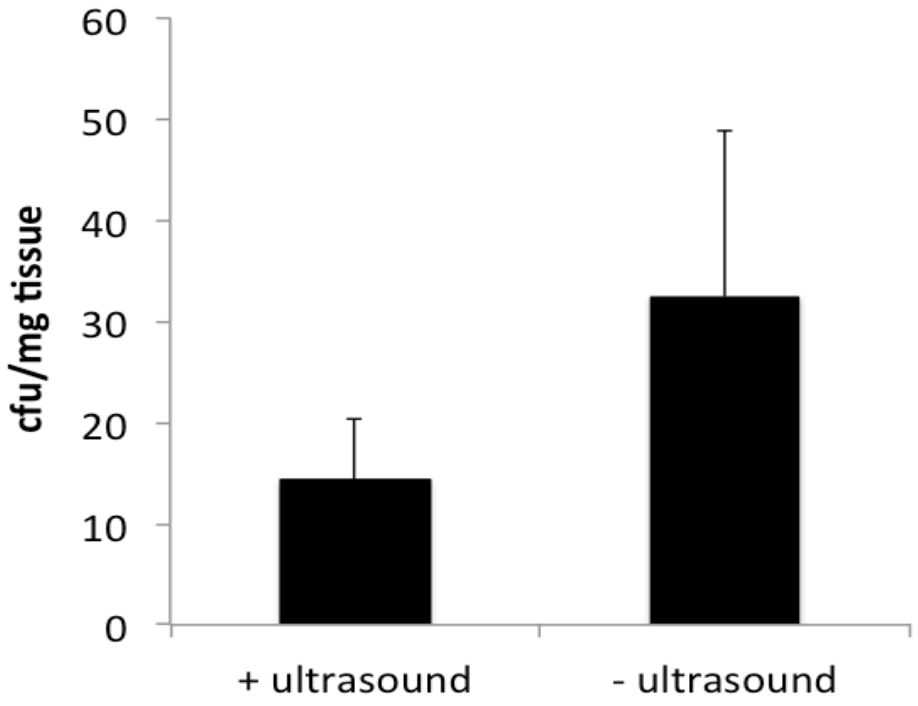
TE skin infected with *S.aureus* (Gram)



TE skin infected with *P.aeruginosa* (Gram)

Shepherd J et al Tissue Eng Part C 15:475 (2009)

At 50kHz, treatment of *S.aureus* both in suspension and in an infected tissue engineered model of human skin are sufficient to substantially reduce numbers of viable bacteria (Shepherd & Rose, unpublished data).

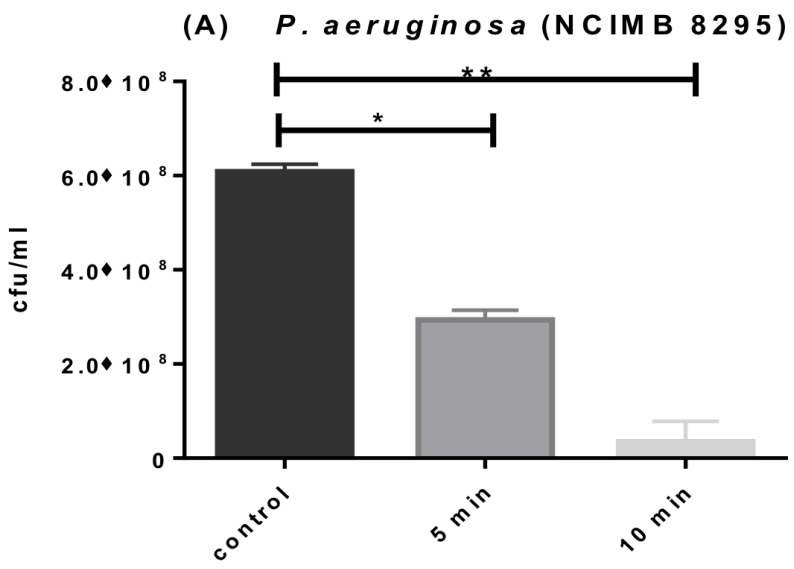


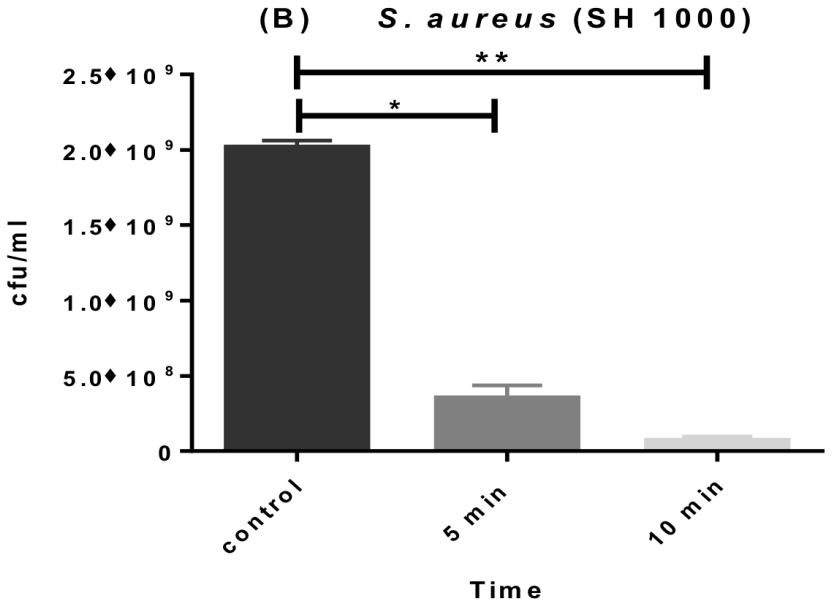
S.aureus infected skin, 50kHz, 5 mins

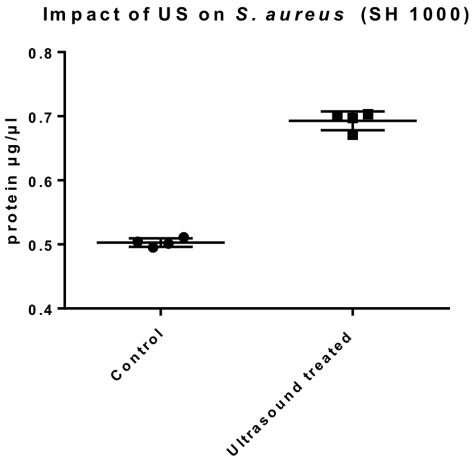
Guma's PhD Aim:

To investigate low frequency ultrasound as antibacterial treatment on planktonic bacteria and bacteria grown as biofilm on TE infected skin model

US at 40kHz kills planktonic bacteria







Protein is released into media following US indicating cell rupture

NEXT: US treatment of infected skin

2) Effects of a novel ultrasound device on wound healing and biofilm

- Project funded by Smith & Nephew
- Dr Toby Holmes
- Using the infected TE skin model
  - Our biofilm is robust
  - Long term effects of US coupling gel on skin

smith&nephew

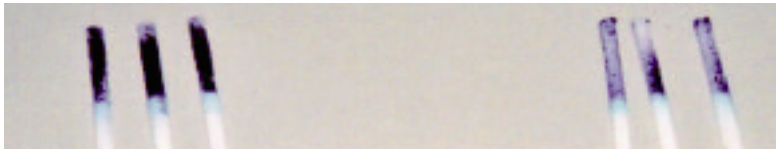
3) ‘Waking up’ bacteria

- Low frequency/low intensity US as a method of making biofilm bacteria more susceptible to antibiotics

- Evidence suggests low frequency, low intensity US might increase bacterial metabolism

S.epidermidis

+ Ultrasound -Ultrasound

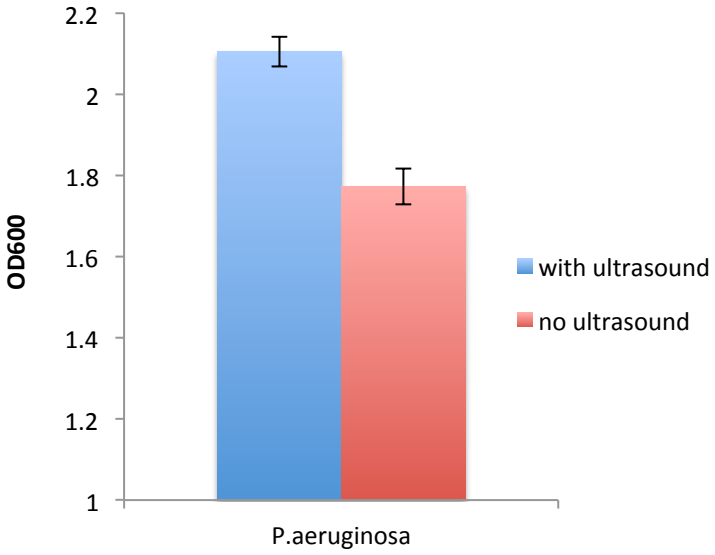


Pitt & Ross, 2003

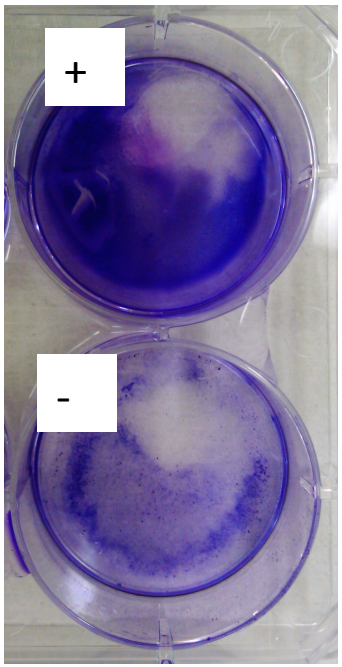
- Can we ‘wake up’ dormant bacteria (‘persisters’) in biofilm so they will be more susceptible to antibiotics?

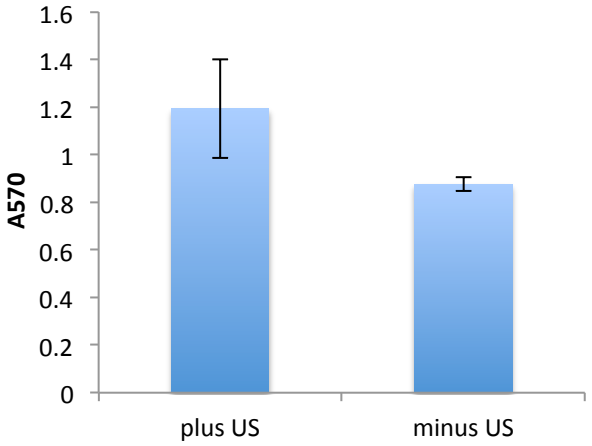
‘LIPUS’ Exogen US bone healing system

20 minute treatment of *P.aeruginosa* in stationary phase with Exogen (pulsed, 1MHz) increases OD600 after 24h by ~25%



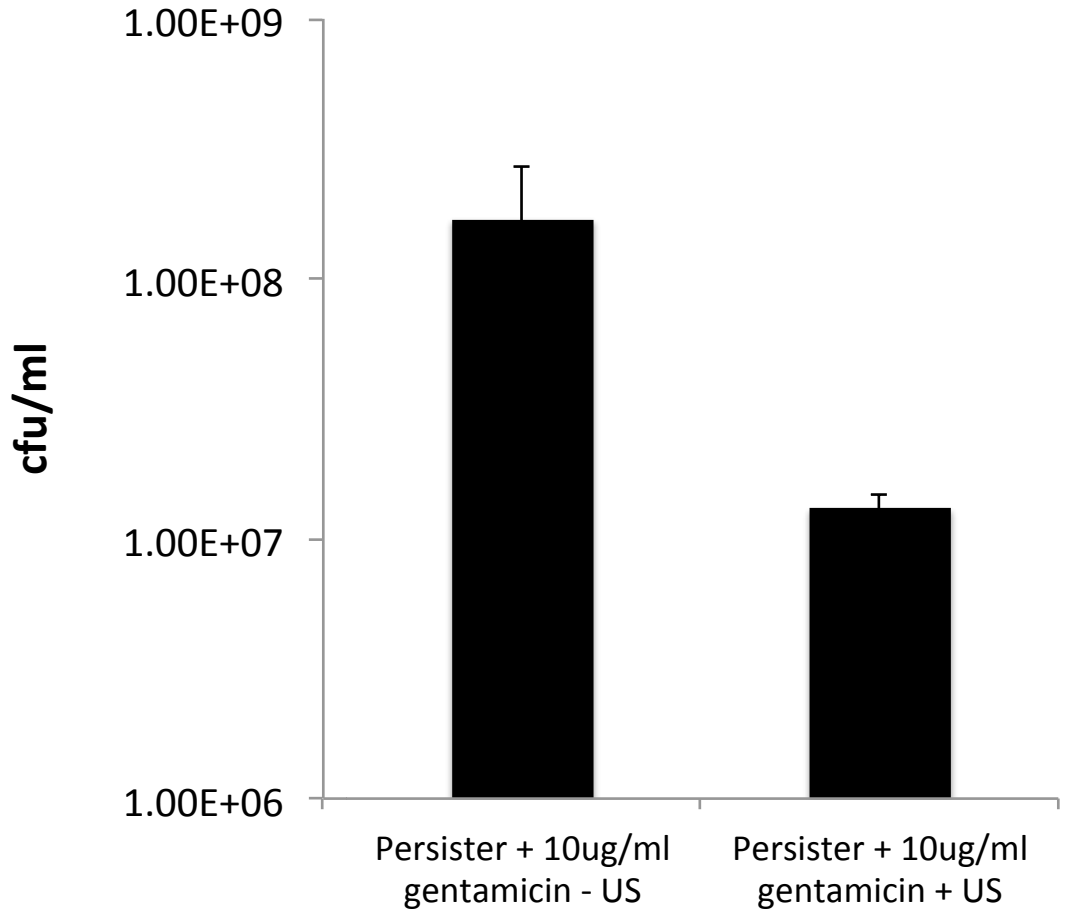
OD (600nm)





Crystal violet

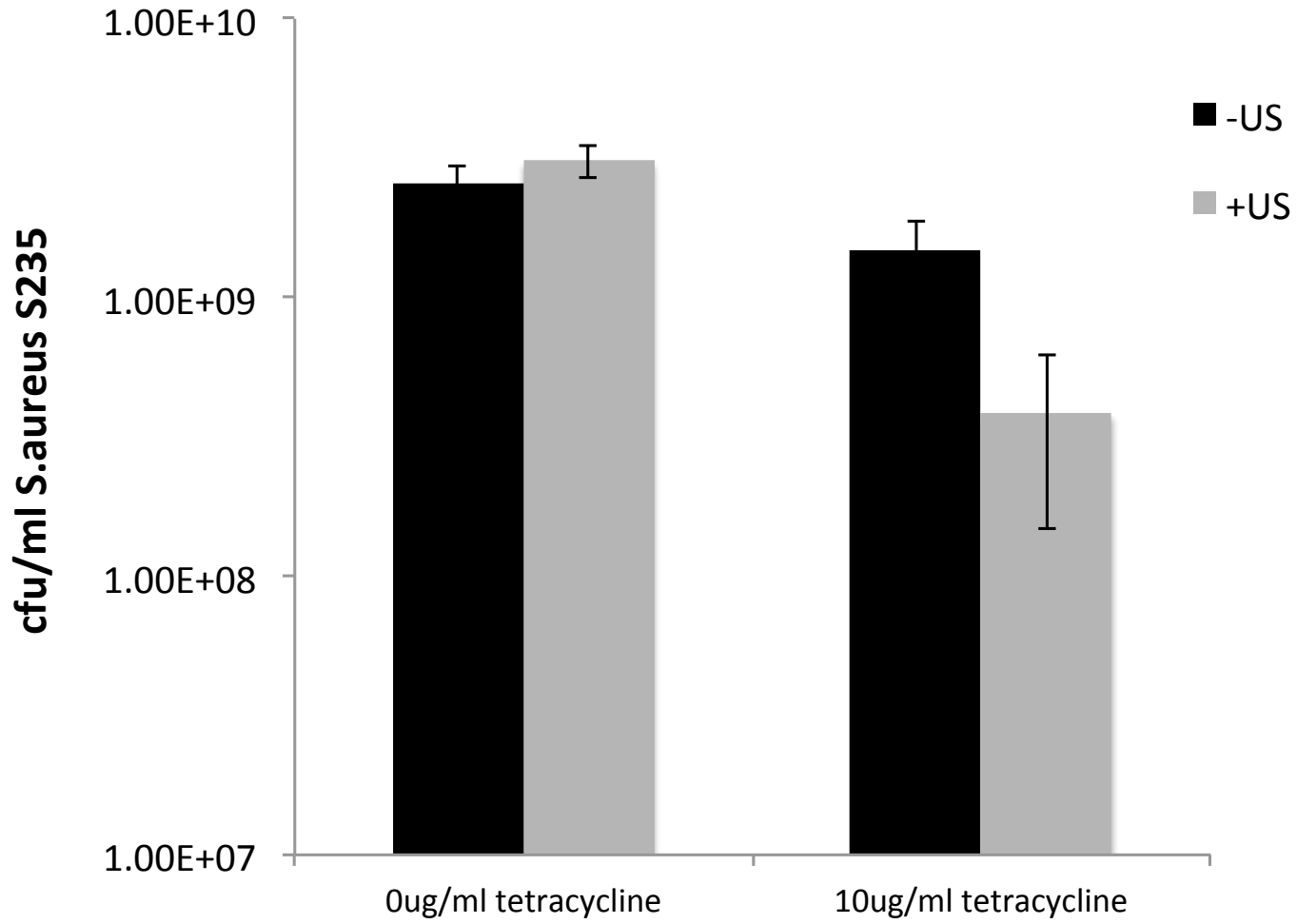
Exogen makes persisters more sensitive to antibiotics



- Persisters more resistant to gentamicin
- Treatment with US makes persisters more susceptible to gentamicin

P.aeruginosa

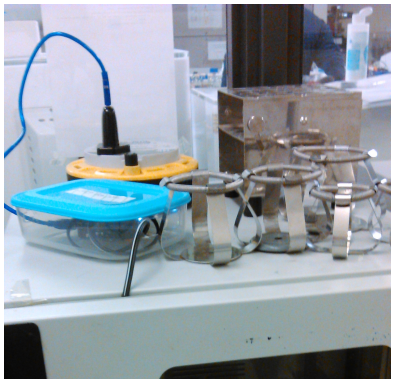
…also effective with *S.aureus*

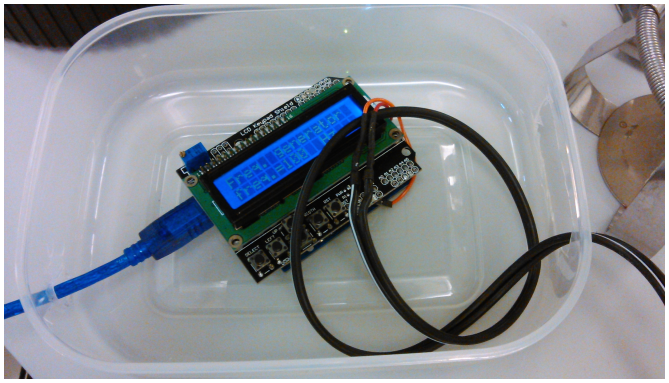


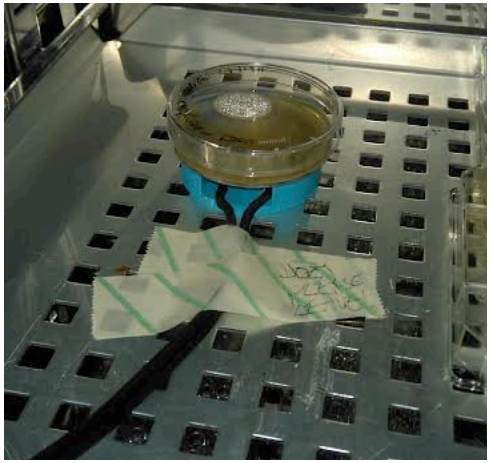
S.aureus S235 persister cells

4) ‘Bug Disco’

Acoustic vibration (not ultrasound!) enhances the formation of *P.aeruginosa* and *S.aureus* biofilm



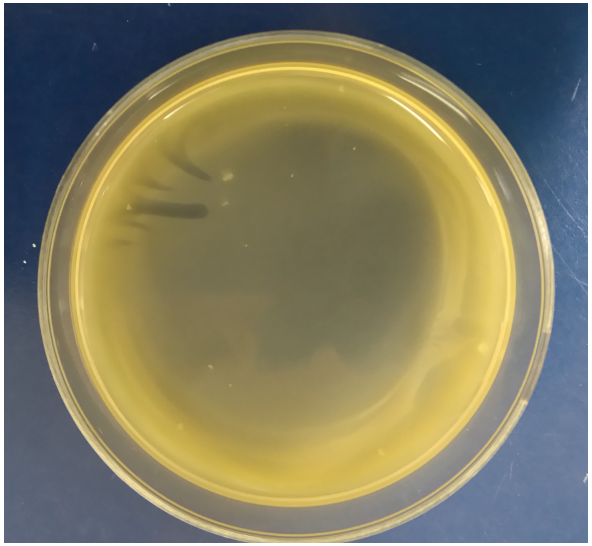




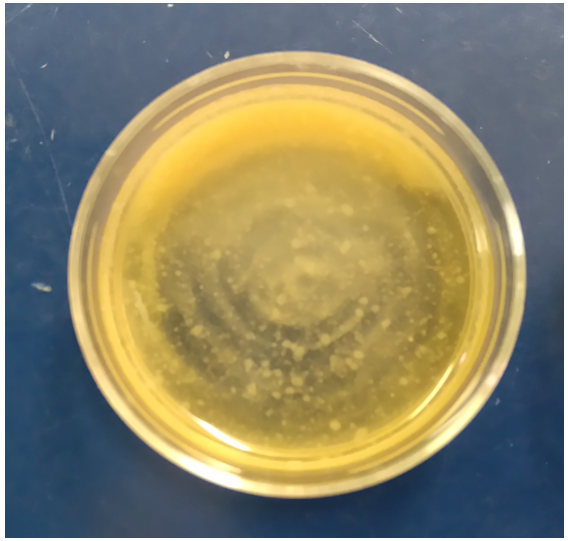
Dr Mark Murphy,
Liverpool John Moores University

Dr Fred Bezombes,
Liverpool John Moores University

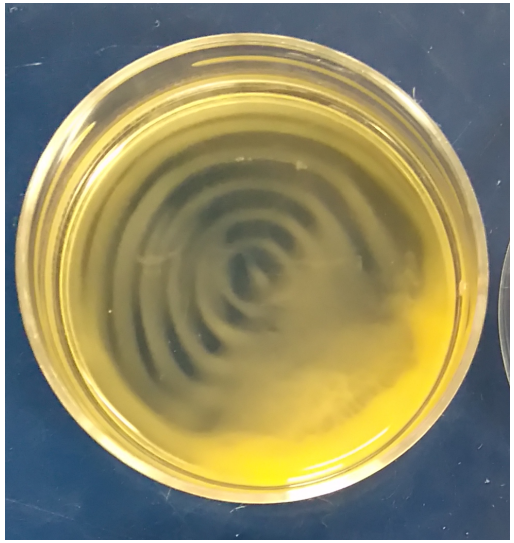
0Hz (*P.aeruginosa*)

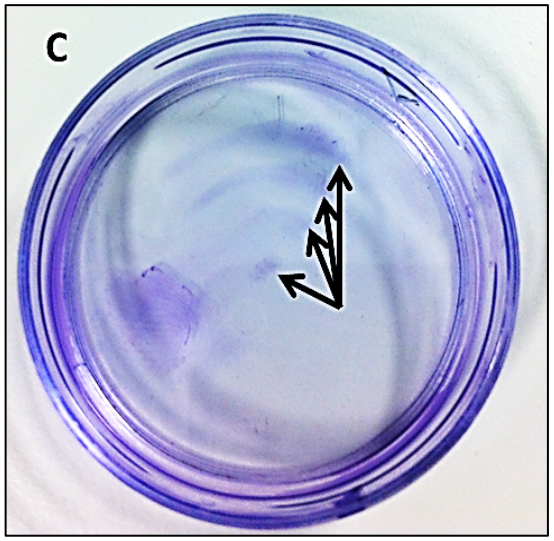


100Hz (*P.aeruginosa*)



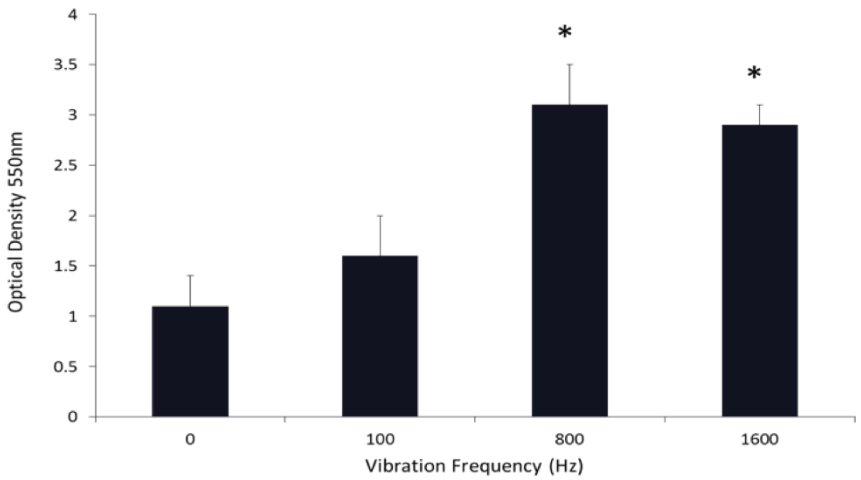
100Hz (*S.aureus*)



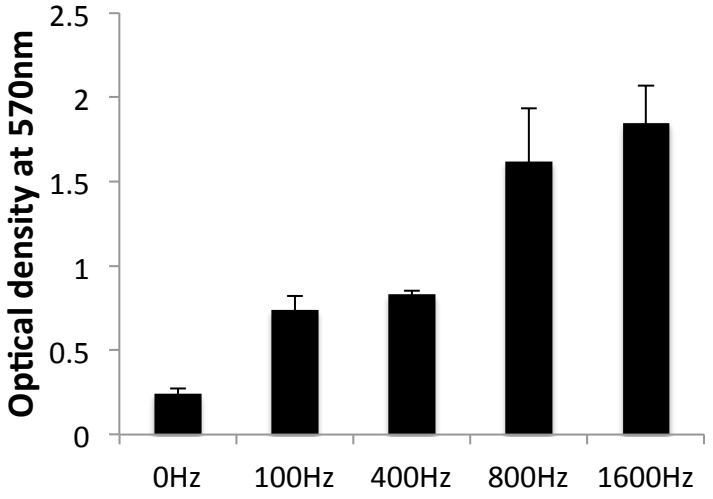


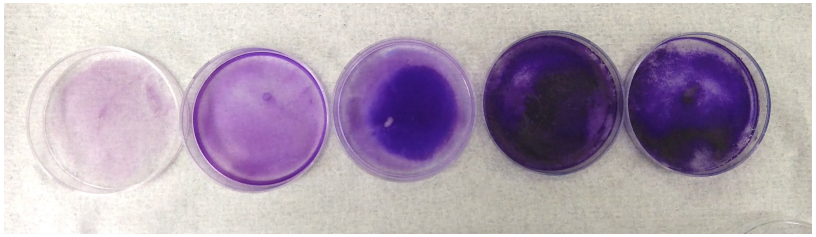
Crystal violet assay - *P.aeruginosa or S.aureus* subjected to acoustic vibration for 48h

P.aeruginosa



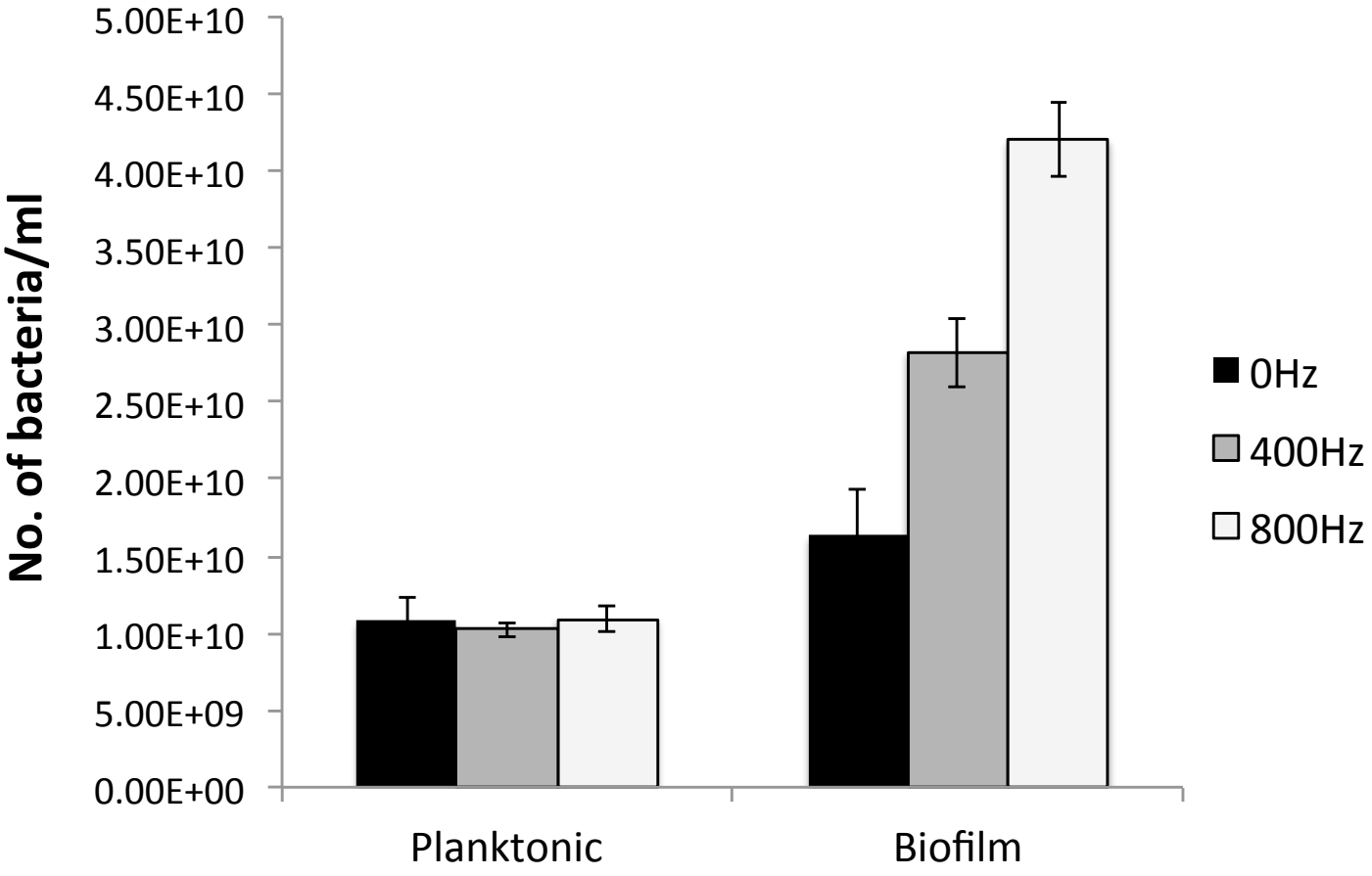
S.aureus





Murphy, Edwards, Hobbs, Bezombes & Shepherd - *(J. Bioscience & Bioengineering* 2016, manuscript submitted)

P.aeruginosa

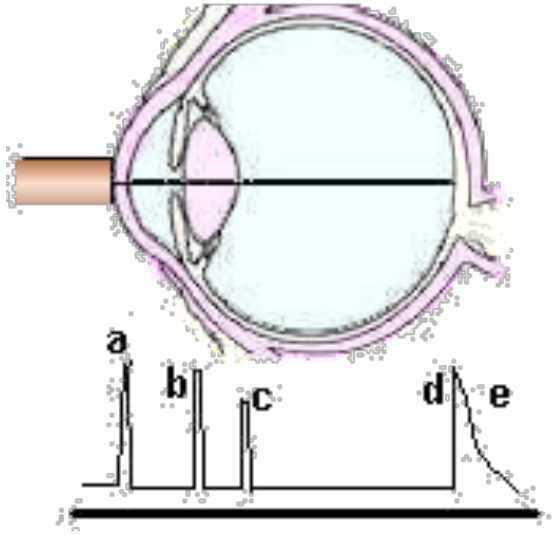


US in Opthalmology

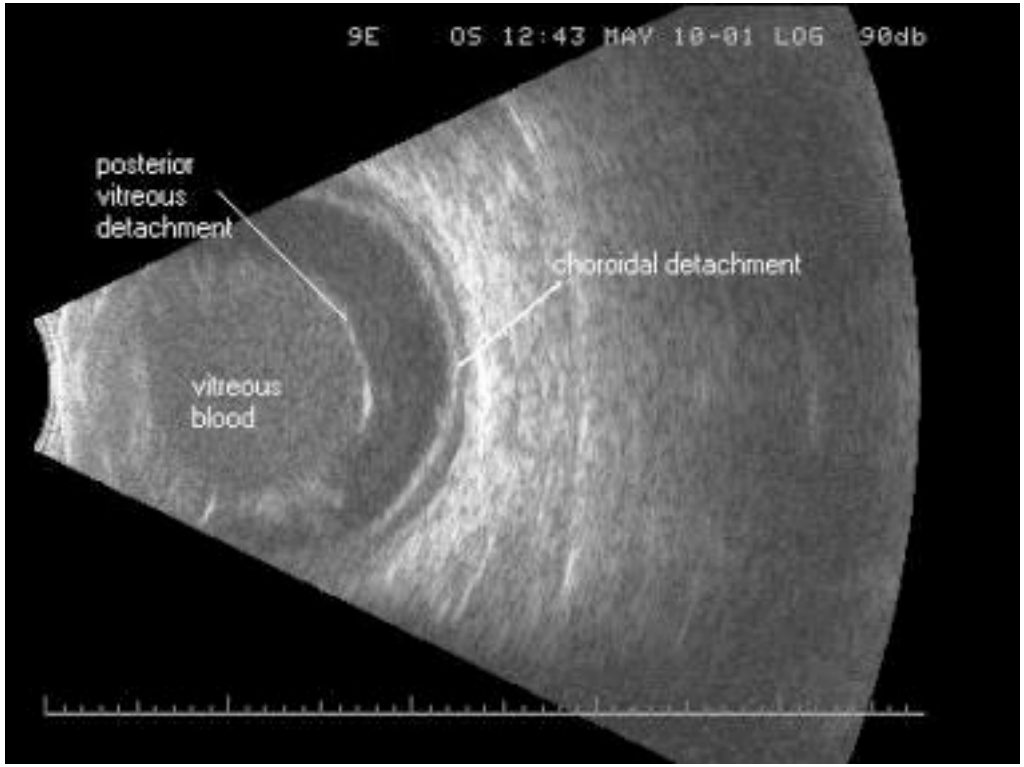
- US mainly used for imaging ocular pathology & anatomy – a superficial fluid filled structure

- Two main types of ultrasound used in ophthalmologic practice:
  - **A-scan:** 8 MHz - obtaining globe length to calculate corrective lens power requirements.
  - **B-Scan:** 10 MHz - Frequently used to examine retina in diabetic patients who have developed vitreous hemorrhage to determine whether they have developed a retinal detachment. Also used for measurement of tumors

- Improved visualization of structures obscured by opaque substances, such as dense cataracts or vitreous hemorrhage.



A scan - routine diagnostic test. Provides data on the length of the eye, a major determinant in common sight disorders.



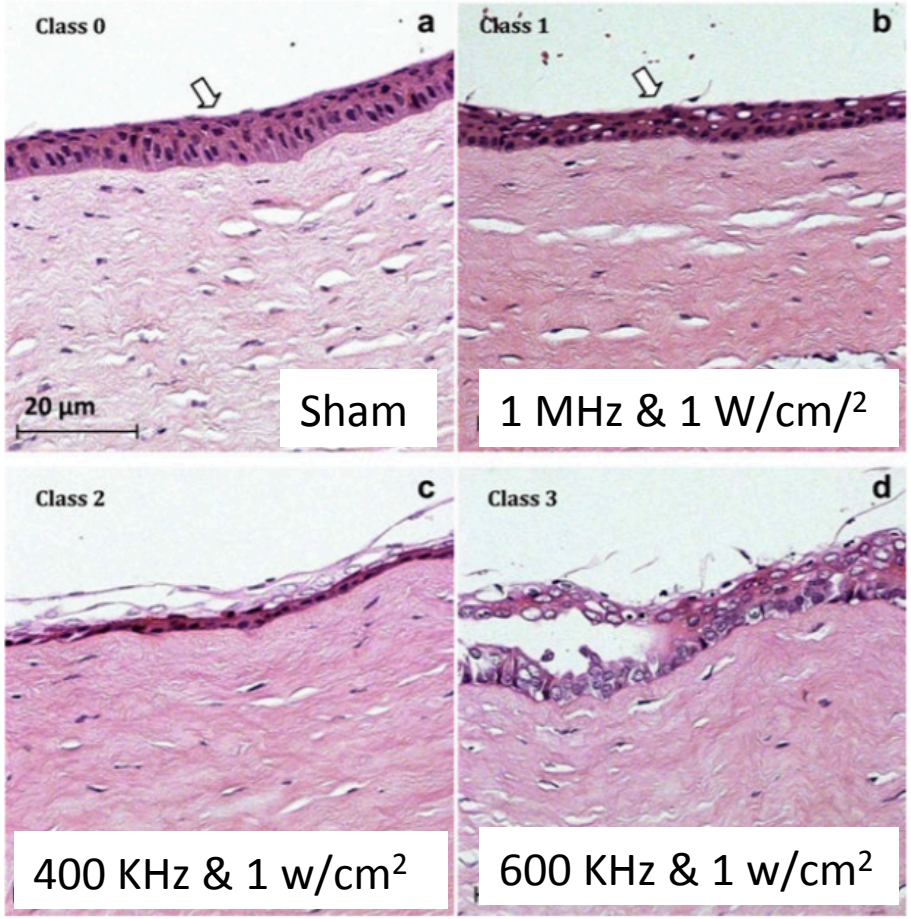
B scan – imaging similar to CT-scan.

Can US be used to enhance delivery of antibiotics into the eye?

- Nabili *et al* (2013) – 5 min treatments of US plus dexamethasone to rabbit corneas to see if US could overcome barrier properties of cornea

- Permeability of cornea to dexamethasone but not tobramycin increased at 400 KHz

- Histological analysis showed structural changes limited to epithelial layers of cornea



Potential of using antimicrobial polymers in US gel?

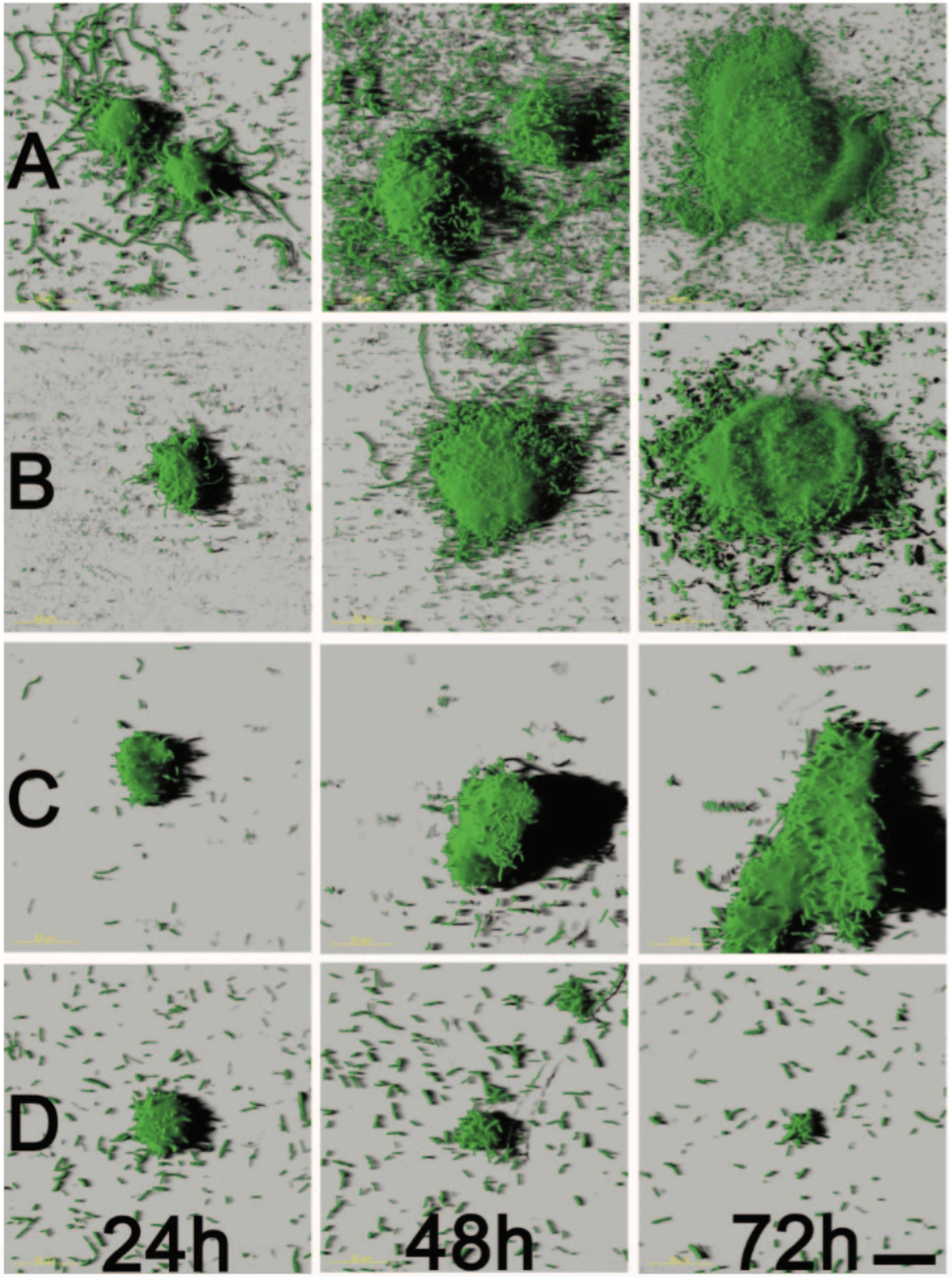
- US gel is bacteriostatic
- Cross-infection is a problem
- Norris *et al* (2005) – designed a drug delivery polymer matrix of pHMEA hydrogel coated with ordered methylene chains which form a US-responsive coating
  - Ciprofloxacin retained within polymer, released on 43 kHz US irradiation

No US
No ciprofloxacin

US
No ciprofloxacin

No US
Ciprofloxacin

US
Ciprofloxacin



Methylene chain-coated pHEMA hydrogel & *P.aeruginosa*

Norris *et al* (2005) *Antimicrobial agents and chemotherapy* 49:4272

Research in progress

- Accoustic waves affect both the amount of biofilm and biofilm patterning according to frequency and amplitude…
- Low frequency high intensity ultrasound reduces viable bacteria numbers in suspension, on an infected TE skin model and also intracellularly within infected ECs….
- Low frequency low intensity pulsed ultrasound may encourage growth of biofilms by increasing bacterial metabolism - making them more susceptible to antibiotics ….

Thank you!

UoS

- Ian Douglas
- Sheila MacNeil
- Craig Murdoch
- Graham Stafford
- Ilida Ortega Asencio
- Tom Paterson
- Toby Holmes

SHU

- Guma Beleid
- Prachi Stafford
- Keith Miller

Smith & Nephew

- Iain Webster
- Tony Dagger
- Dan Fitzgerald

LJMU

- Mark Murphy
- Frederic Bezombes
- Tom Edwards

Sheffield Antimicrobial Resistance Network
SHAMROK

smith&nephew

The University Of Sheffield.

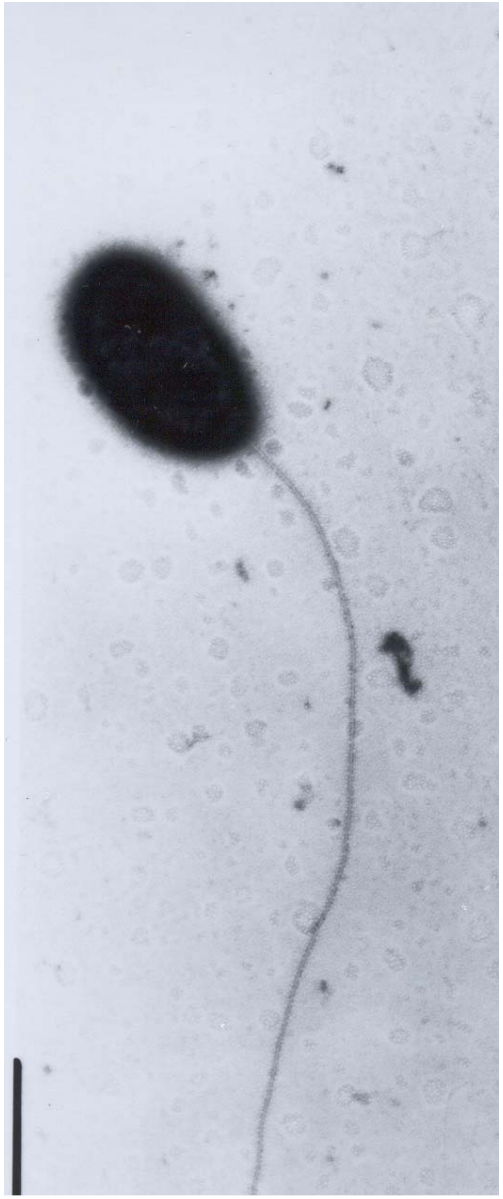
Sheffield Hallam University

LIVERPOOL JOHN MOORES UNIVERSITY

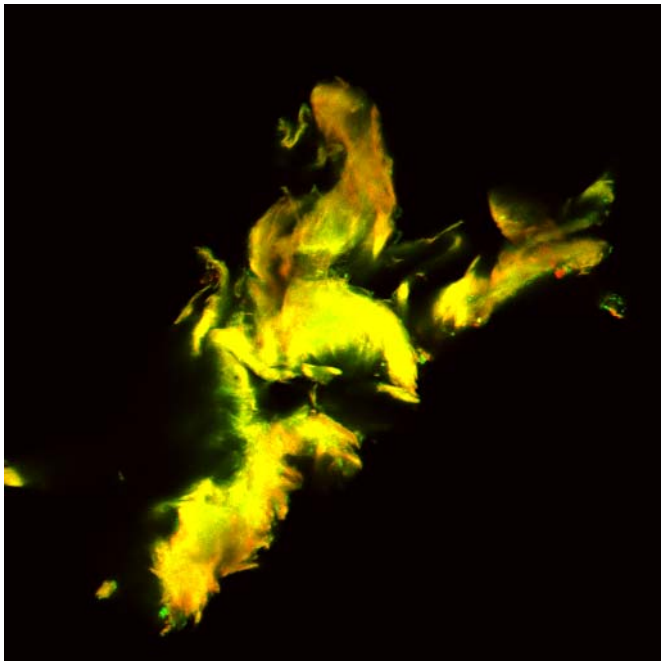
Quorum sensing and antibiotics tolerence

Subhadeep Chatterjee
Staff Scientist
Laboratory of Plant Microbe Interaction
Centre for DNA fingerprinting and Diagnostics
(CDFD), Hyderabad

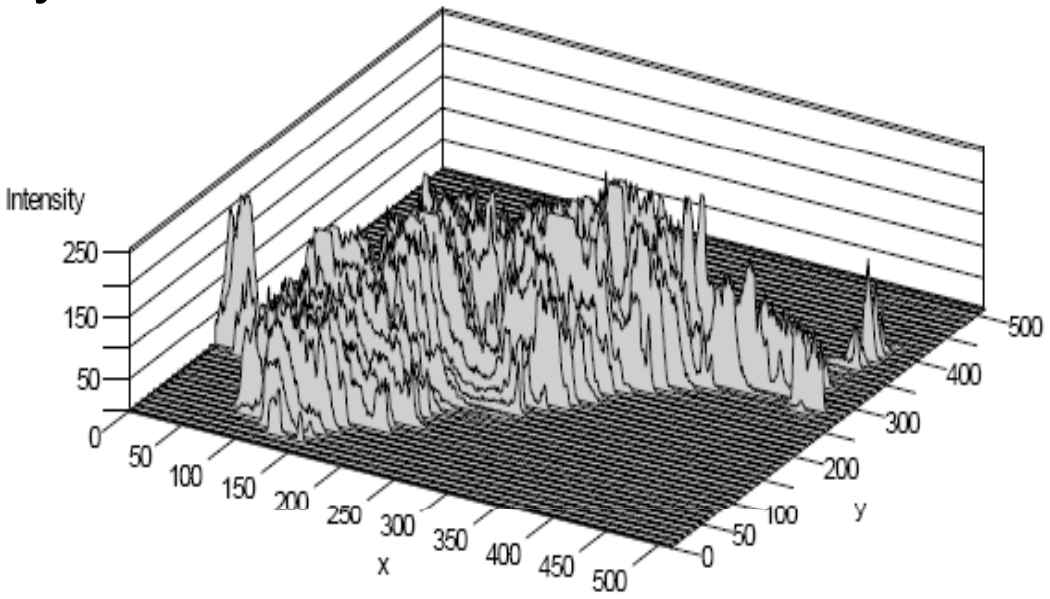
- Quorum sensing.
- How quorum sensing contribute to antibiotics tolerance.
- Heterogeneity in quorum sensing.
- Targeting social behavior of microbes: social drugs.



Community

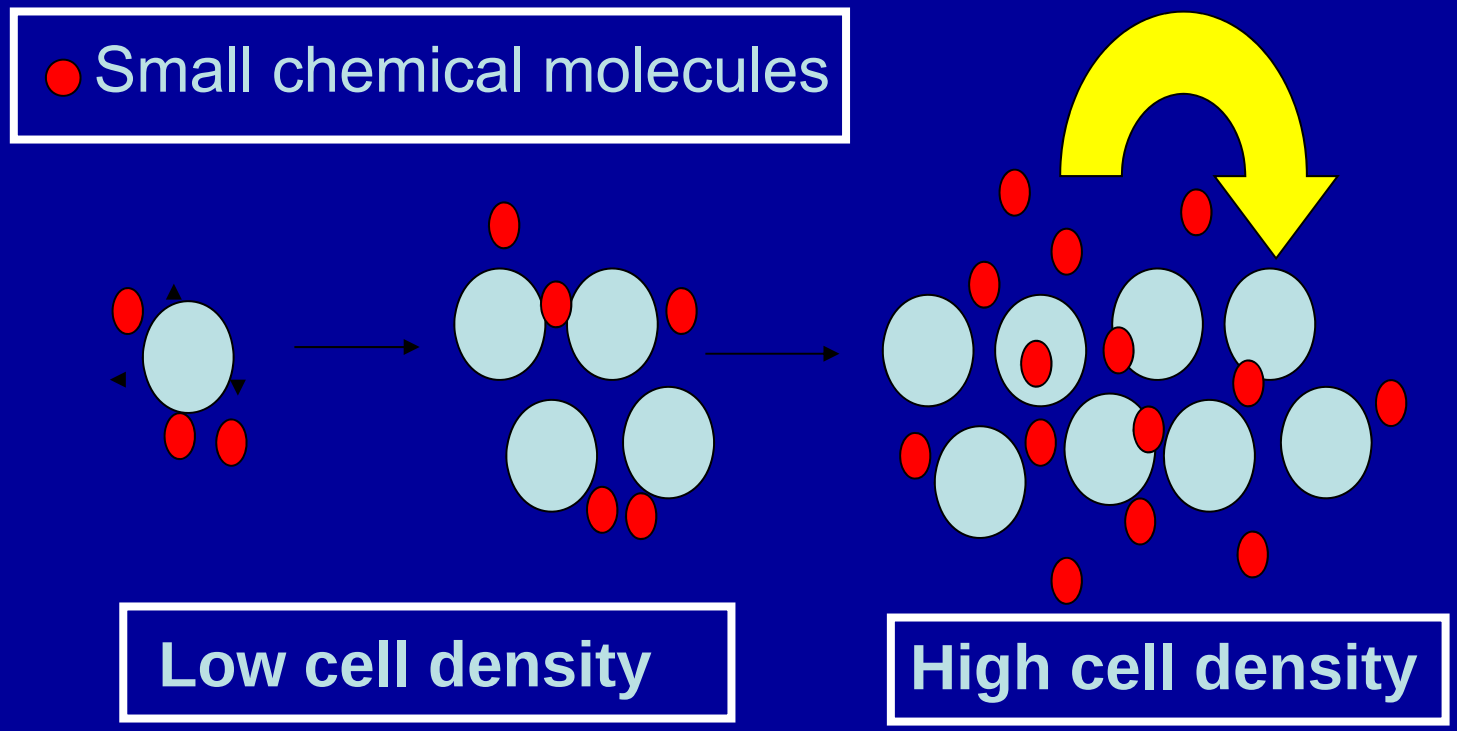


Solitary



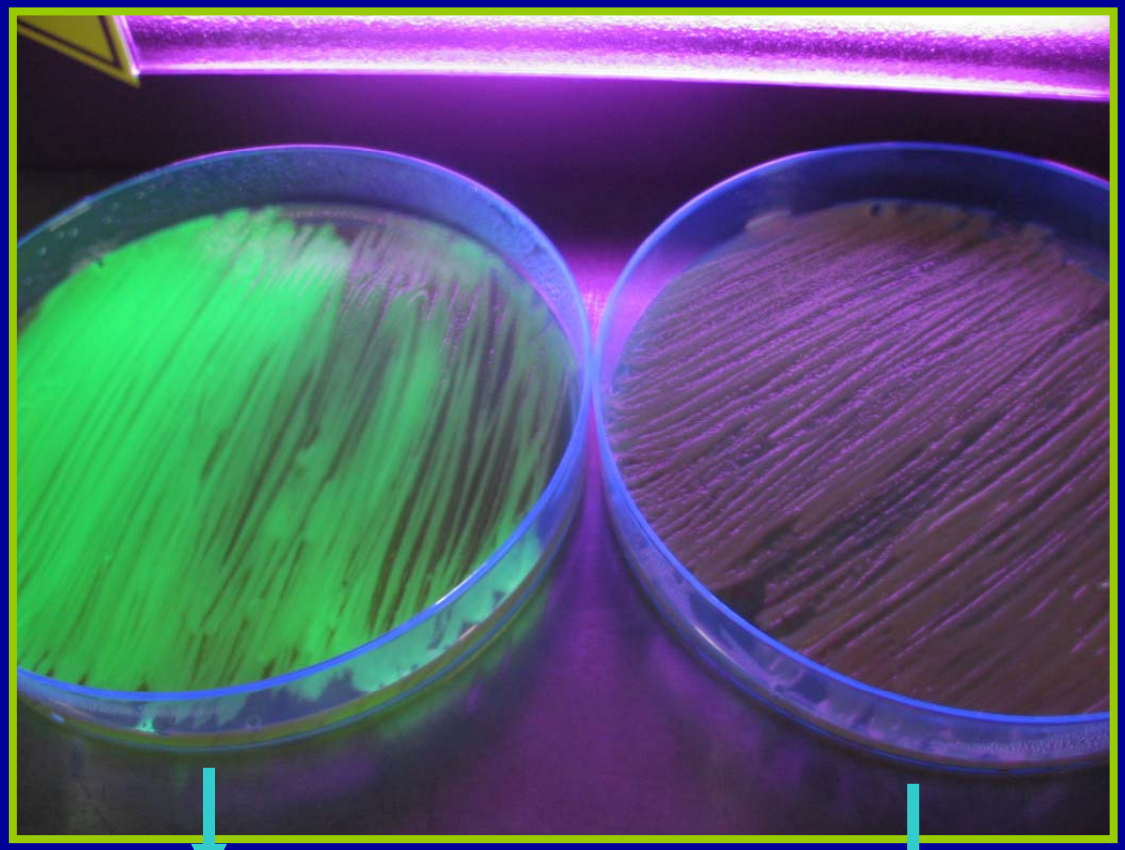
Sociomicrobiology

In prokaryotes, quorum sensing is called as density dependent cell-cell signaling



Bacteria can count and recognize each other by their language

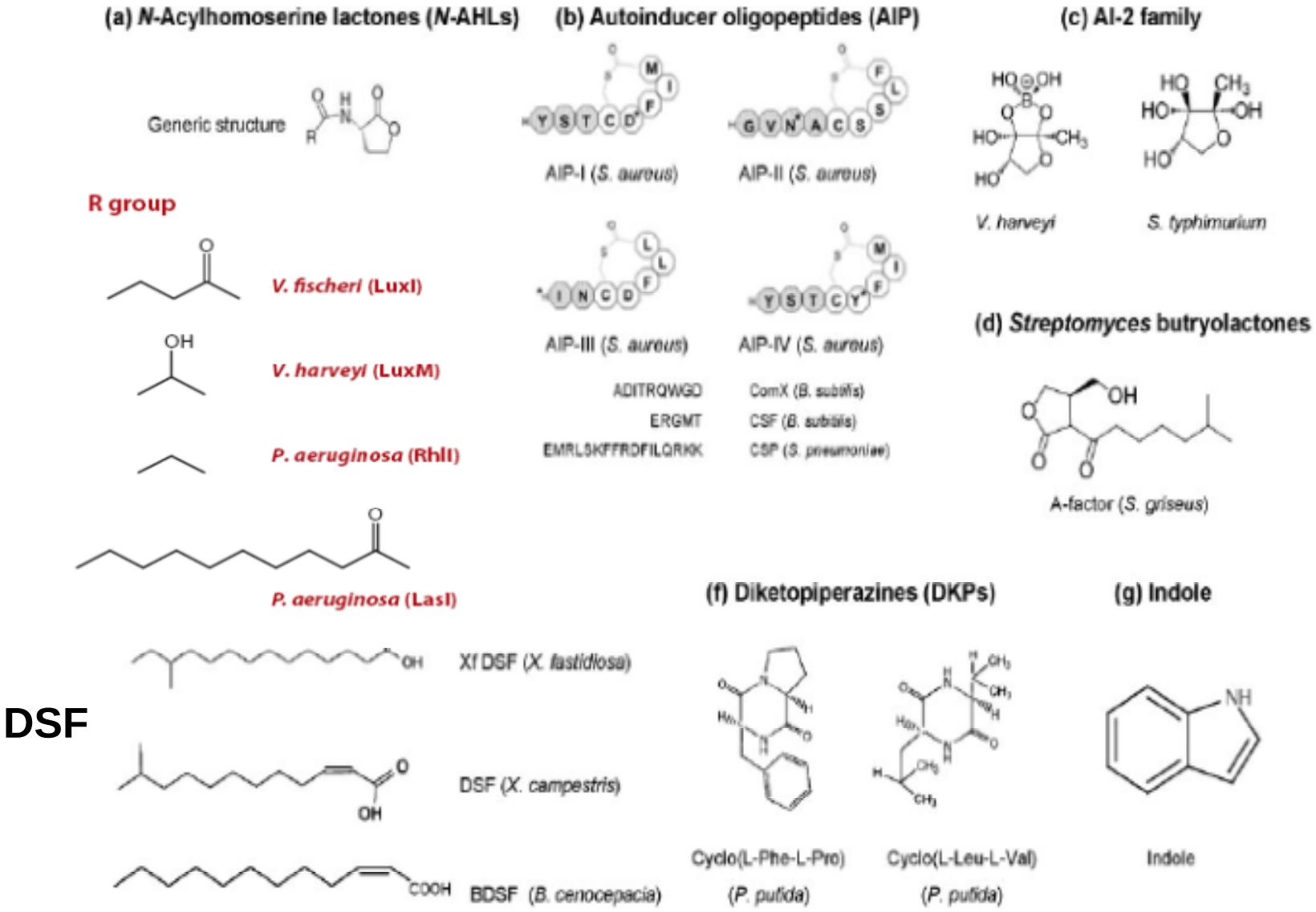
Bacteria love to talk like human



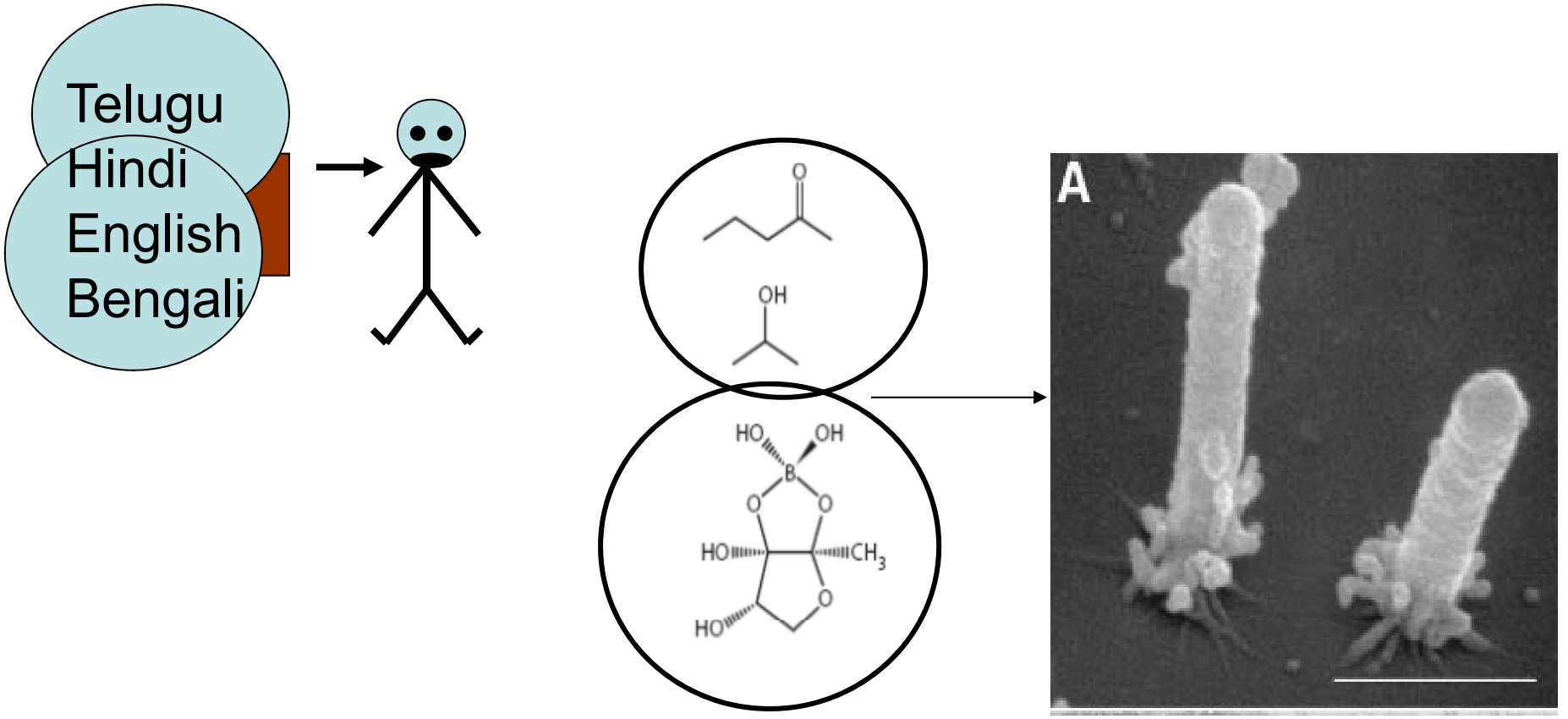
Bacteria making chemical signal and talking with each other

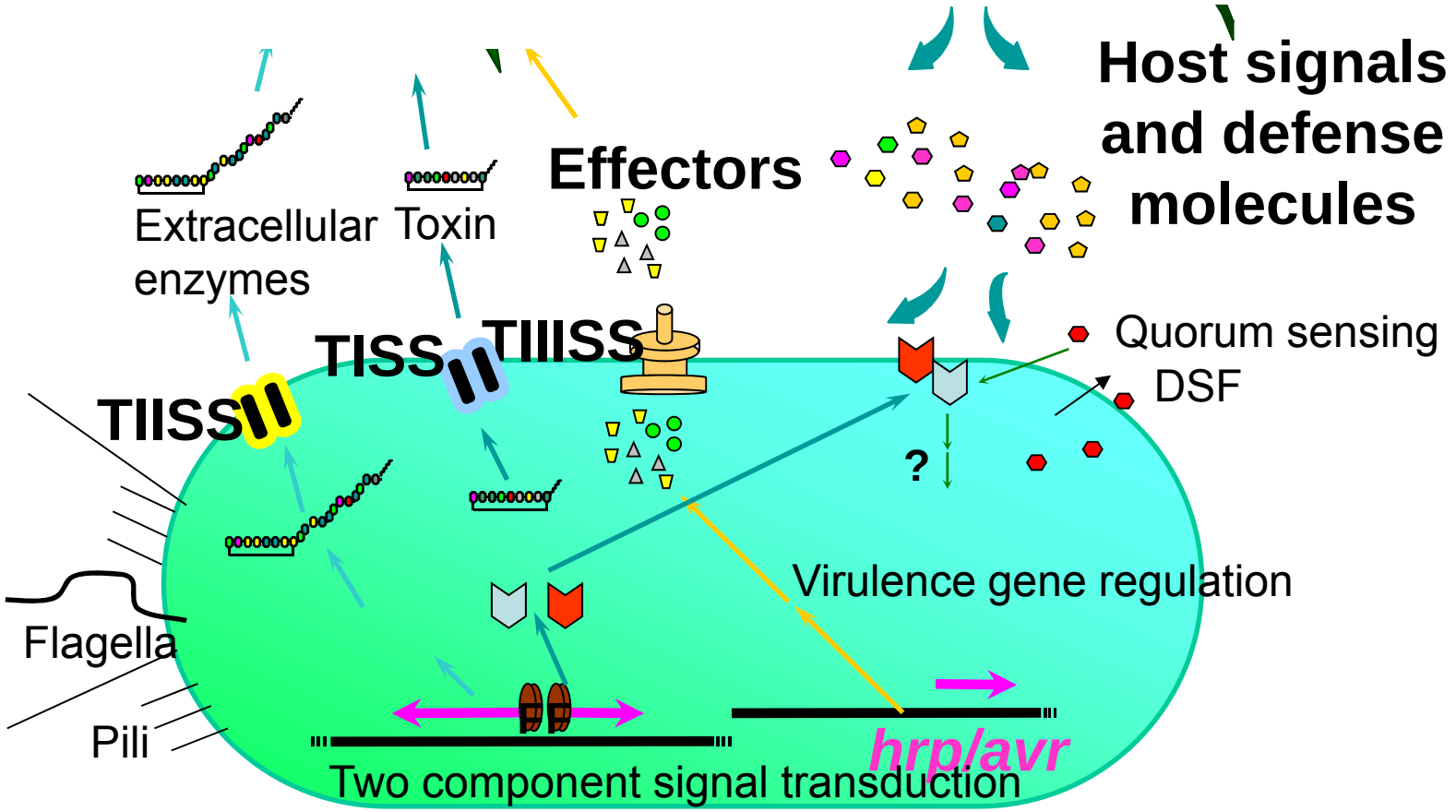
Bacteria which is not able to speak

Bacterial language molecules

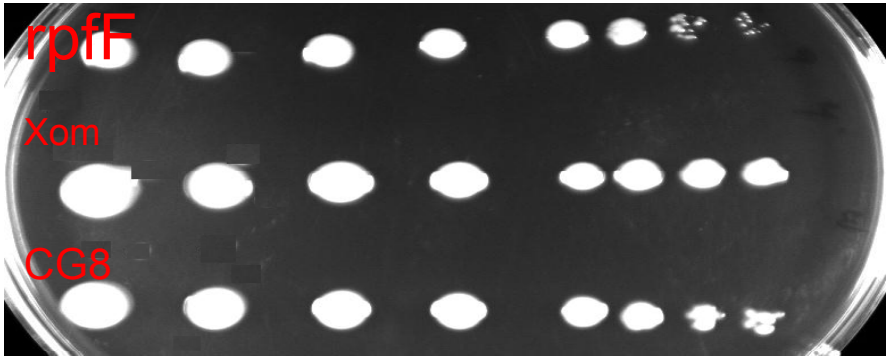


Sometimes, bacteria can speak in two or more different languages

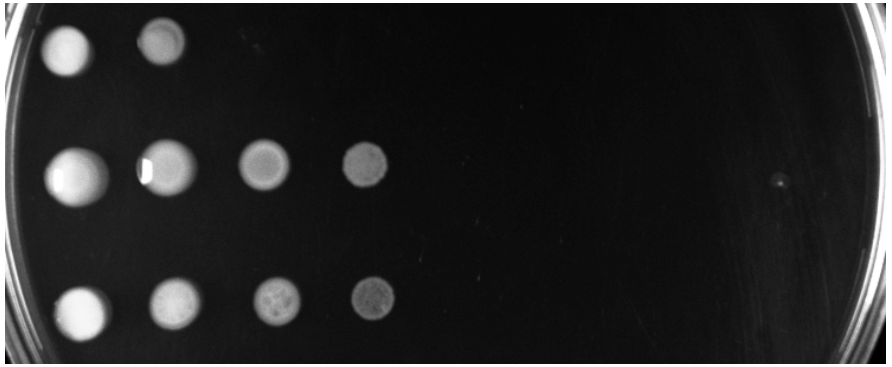



Host signals
and defense
molecules
Effectors
Extracellular
enzymes
Toxin
TISS
TIIISS
TIISS
Quorum sensing
DSF
?
Virulence gene regulation
Flagella
Pili
hrp/avr
Two component signal transduction

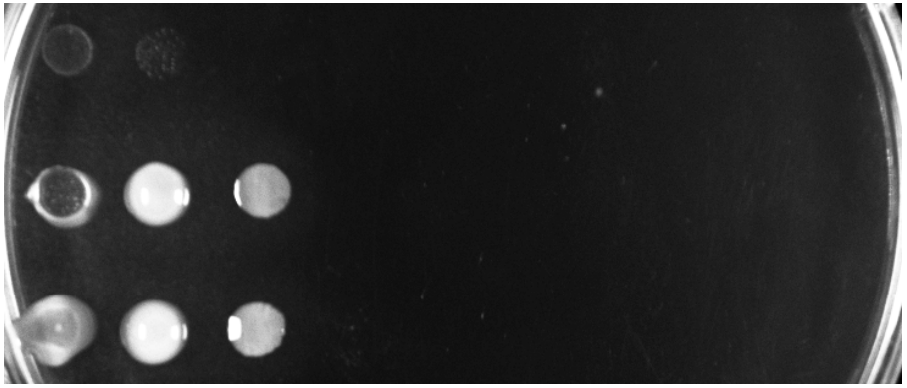
PSA



PSA+1mM Acetosyringeone

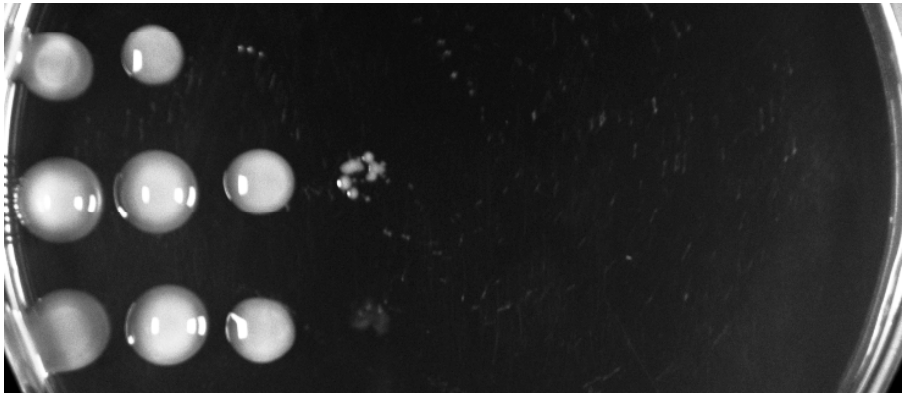


PSA+0.1 % Tween 20

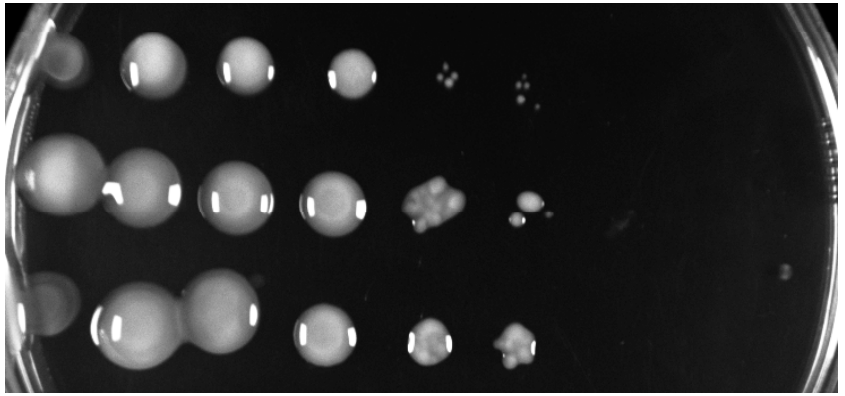


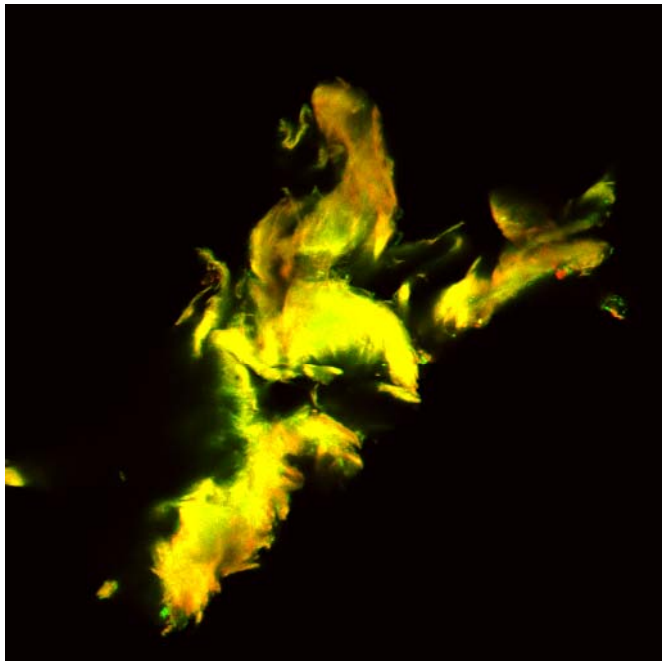
PSA+0.2 %Triton x 100

PSA+ 0.01 % SDS

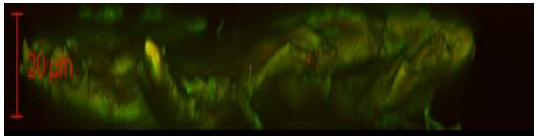


PSA+10mM NaCl

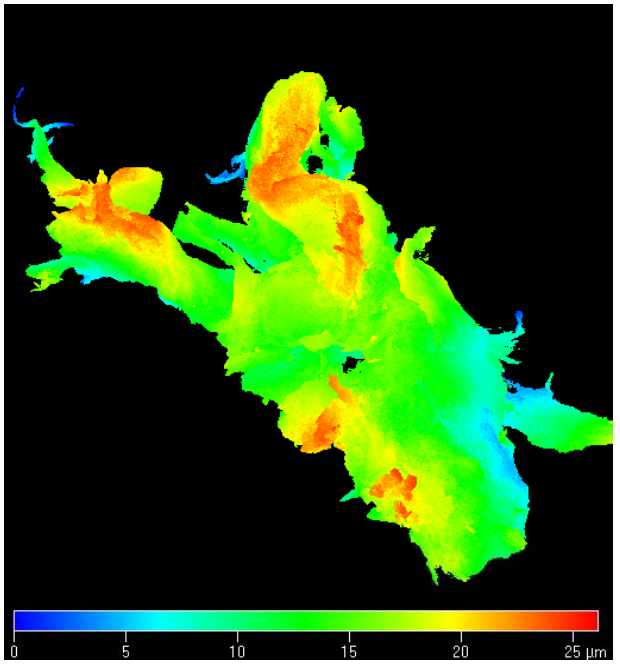


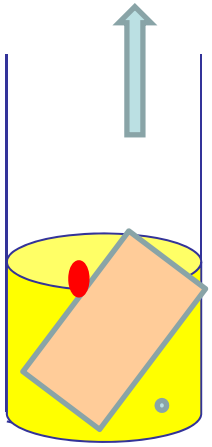


Xoo 24 h

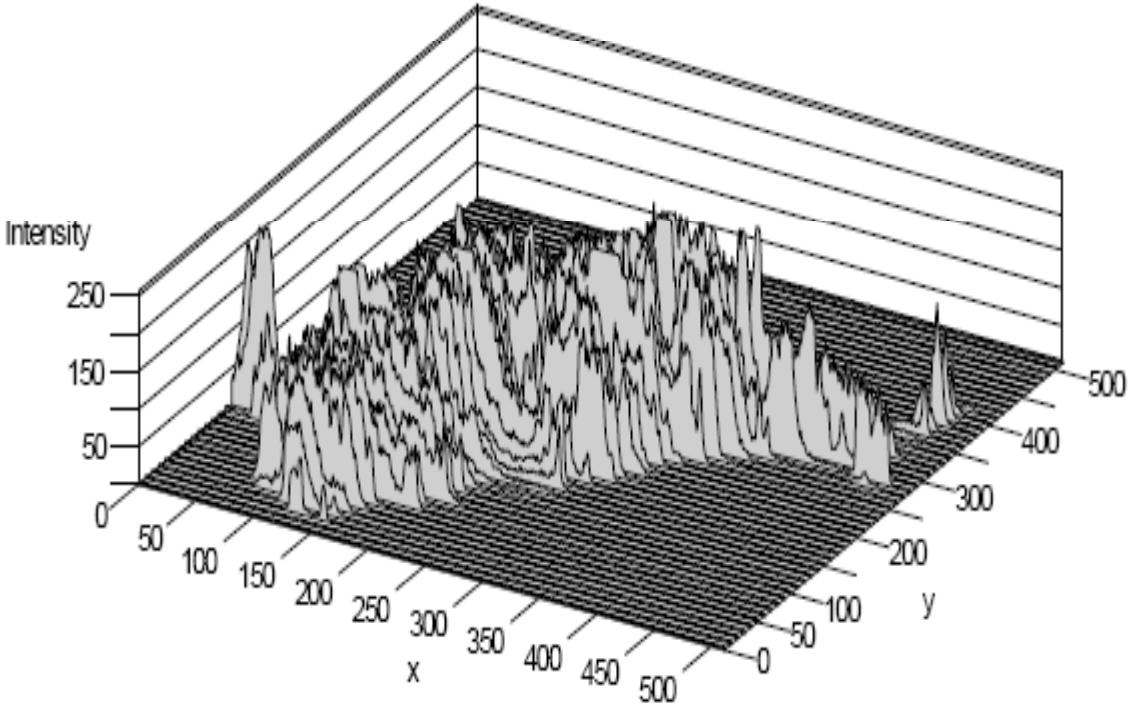


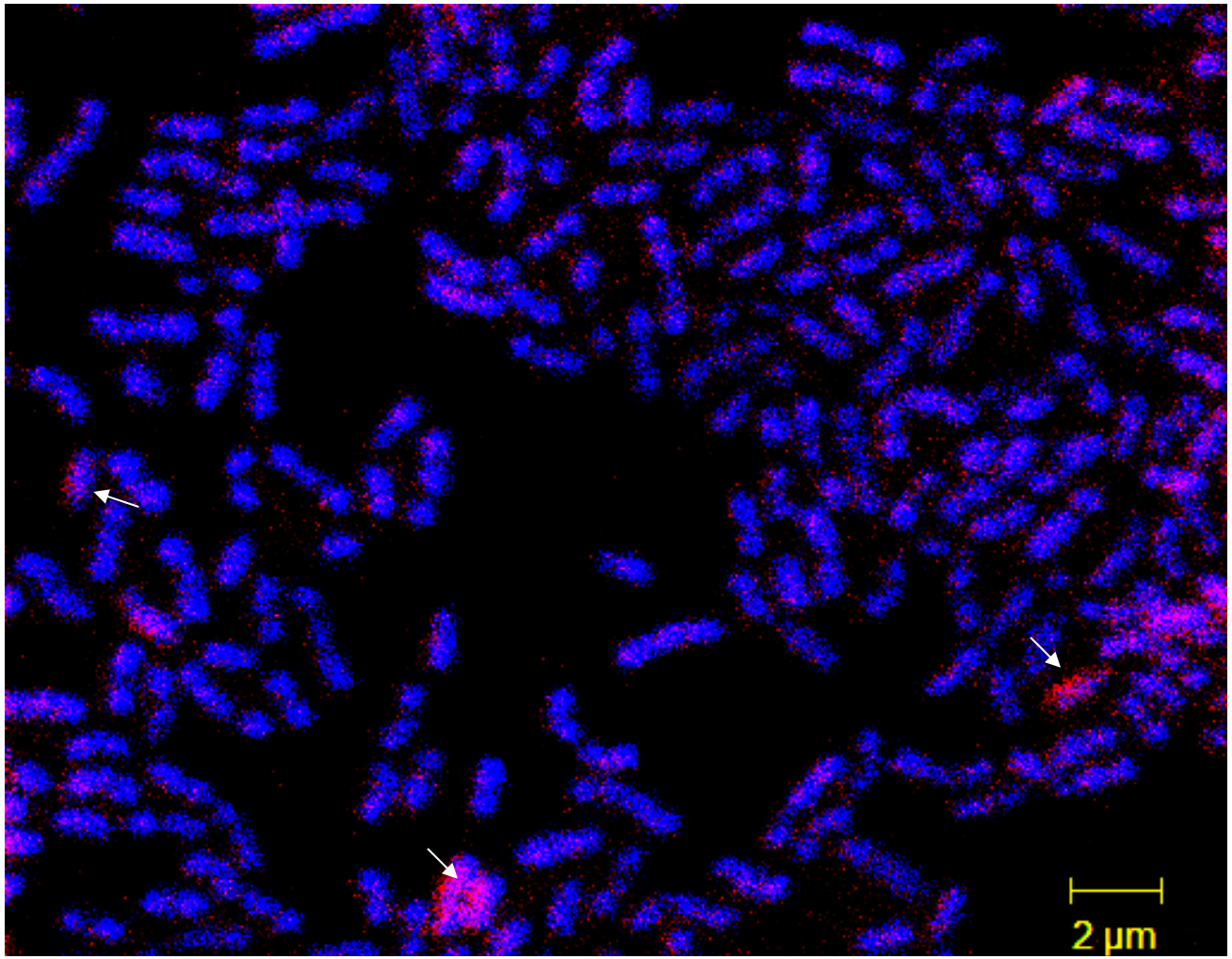
2.5D View



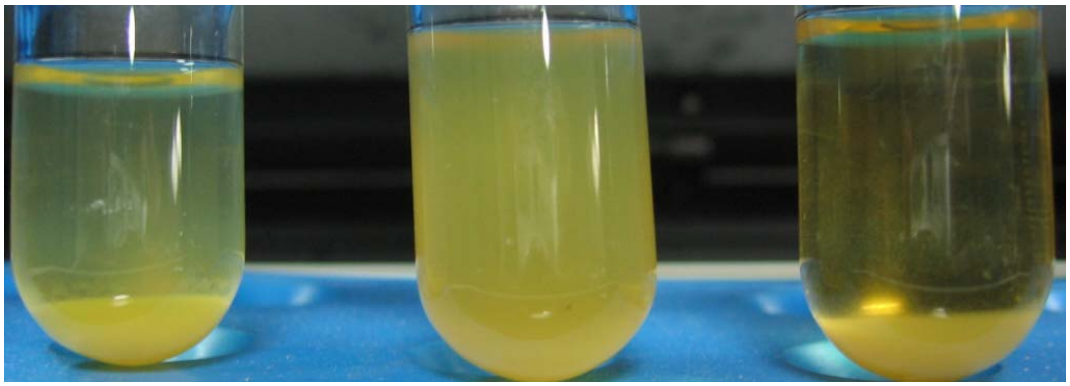


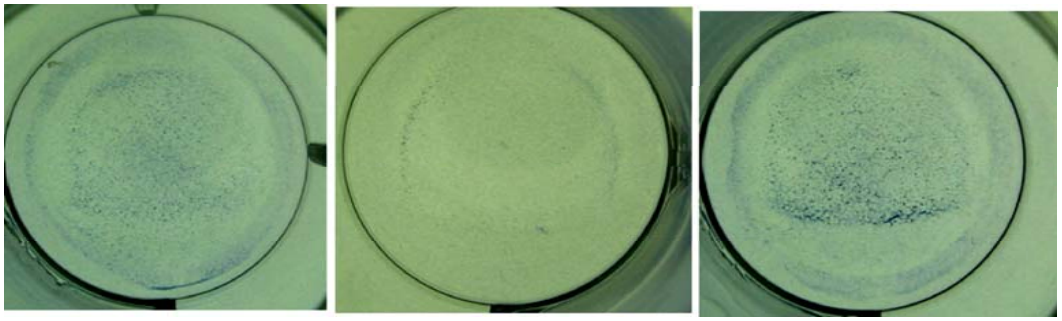
Biofilm formation in
air- media interphase

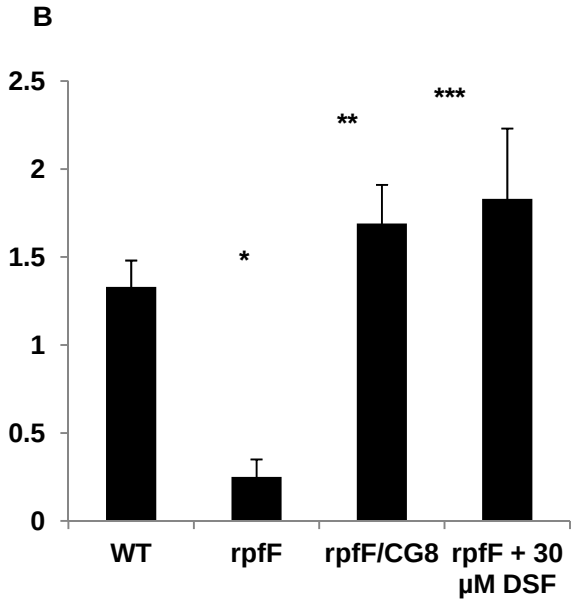


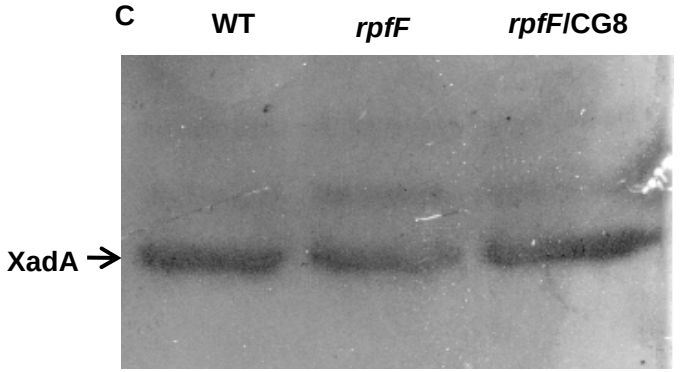

2 µm

DSF promotes attachment and biofilm formation



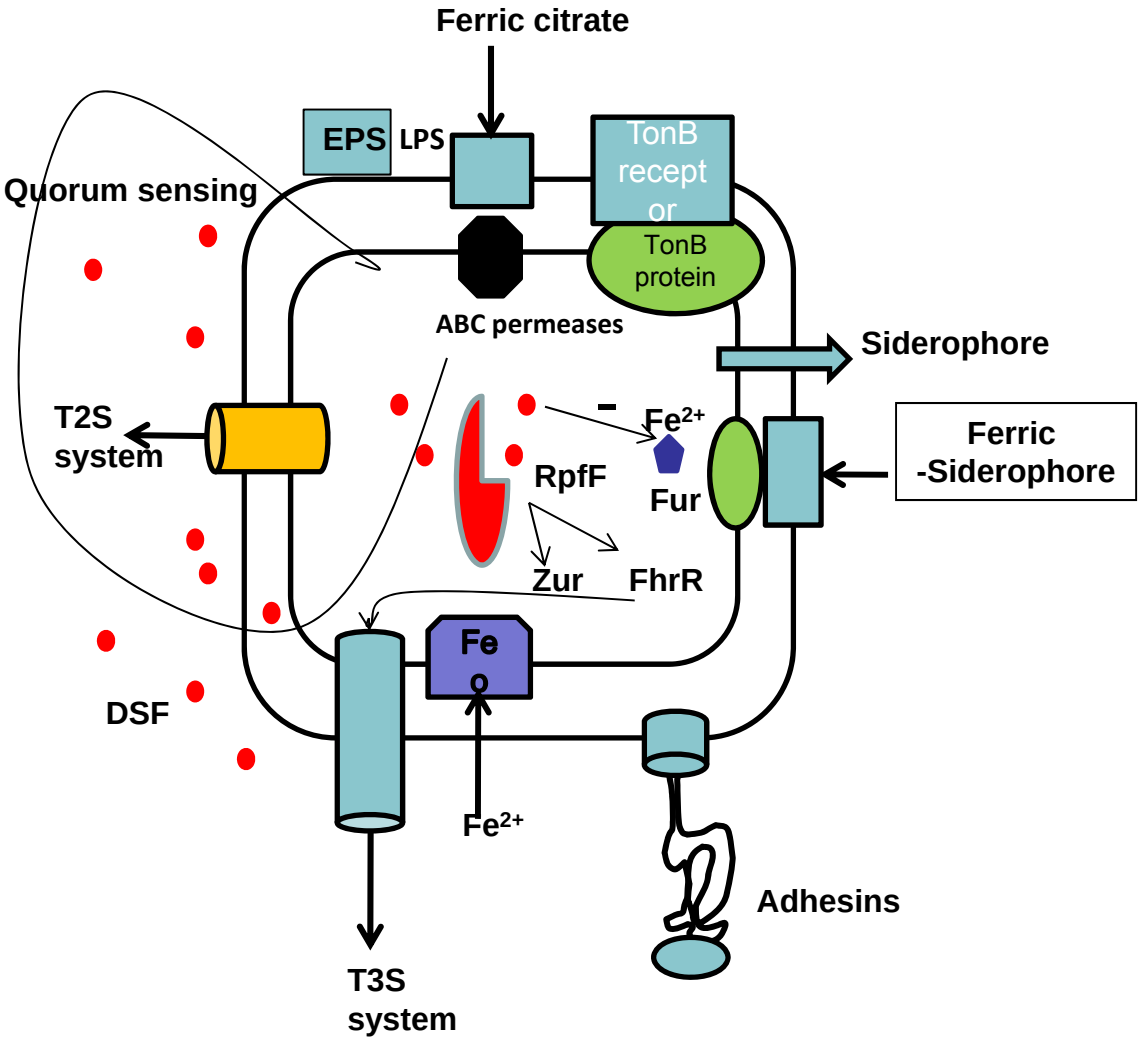






DSF positively regulates adhesins: XadA, YapH, FimA

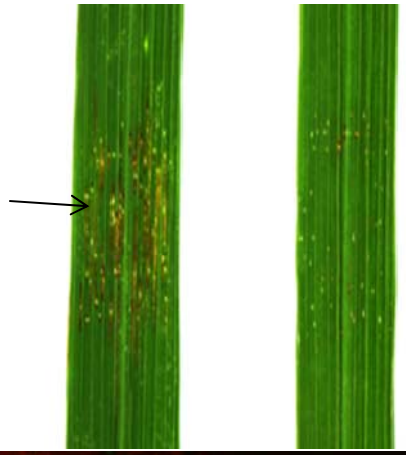
Integration of iron and quorum sensing in *Xanthomonas oryzae*

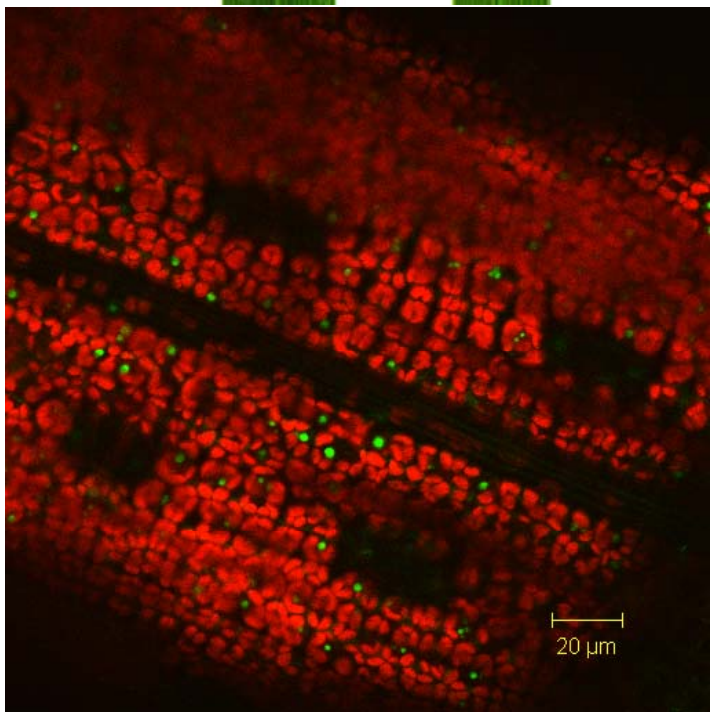


Rai et al., Molecular Microbiology, 2015.

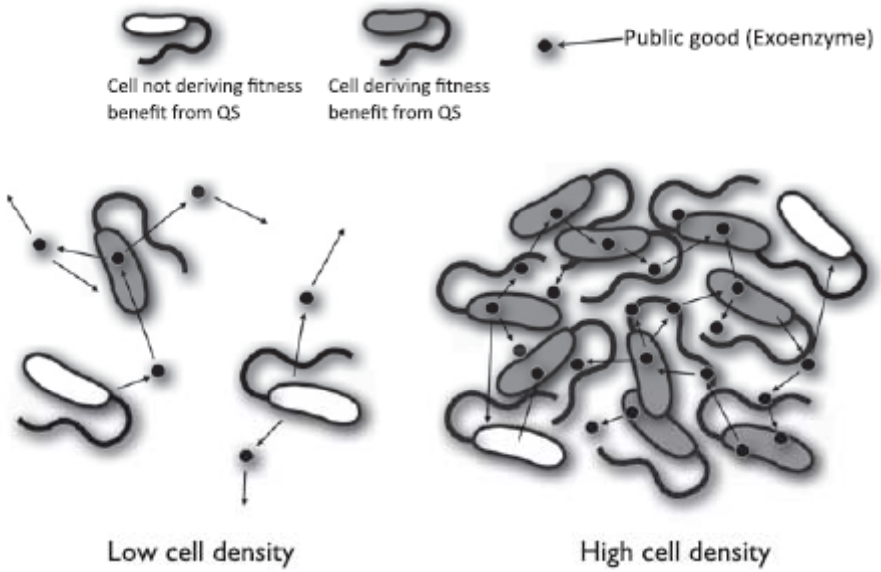
NON-VASCULAR PATHOGEN

Symptoms of Bacterial Leaf Streak caused by *X. oryzae* pv. *oryzicola*





Quorum sensing: Density dependent fitness benefit



Altruism ⟶ **Conflict**

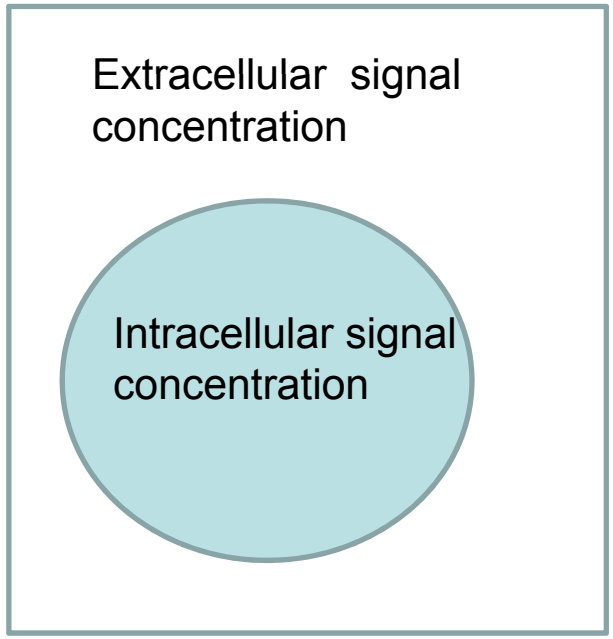
‘Public goods’
‘Private goods’

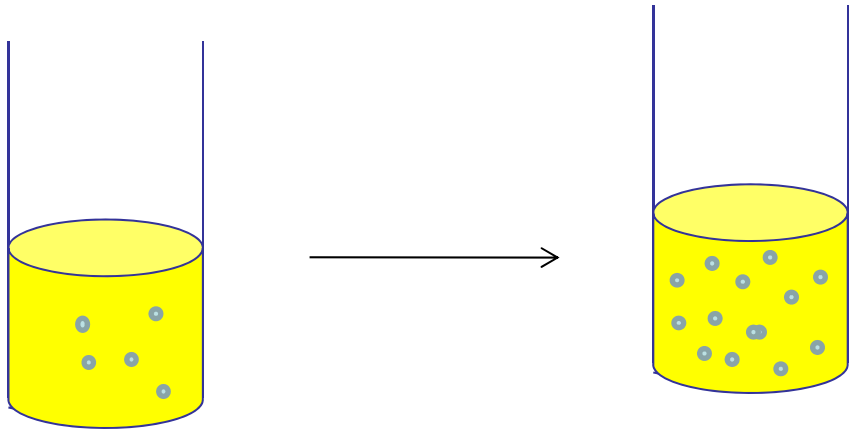
Social behavior:
Exo enzymes
Biofilm
Adhesin
Antibiotic tolerance
motility

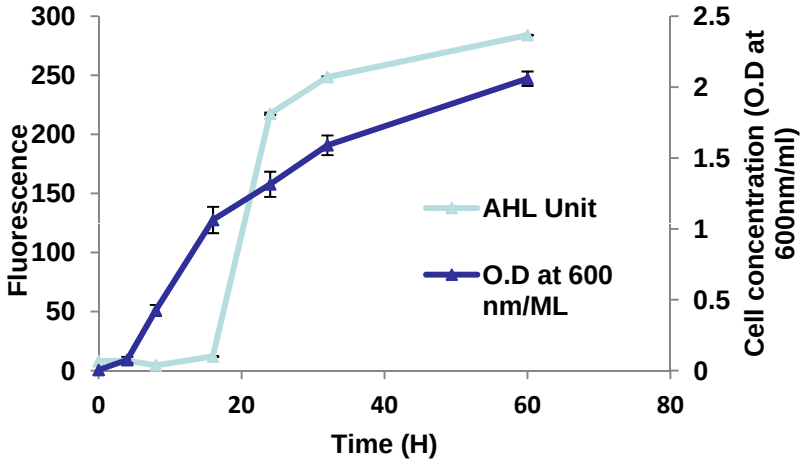
QS synchronizes cells in the population to perform collective social tasks in unison which maximize the benefit at the inclusive fitness of individuals

Phenotypic heterogeneity in performing social tasks is advantageous as it can serve as a bet-hedging survival strategy under changing environmental conditions.

Do bacteria exhibit bet-hedging in QS?





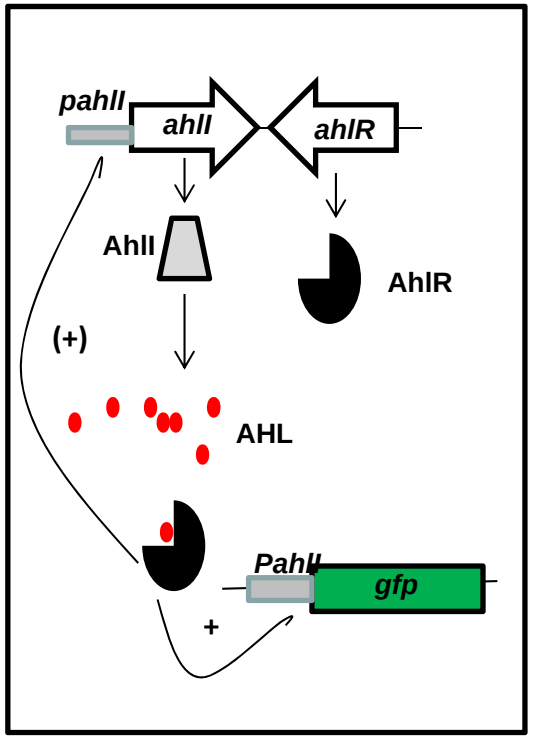


Dynamics of QS response of single cells in the population:

How individual cells behave in QS activated population?

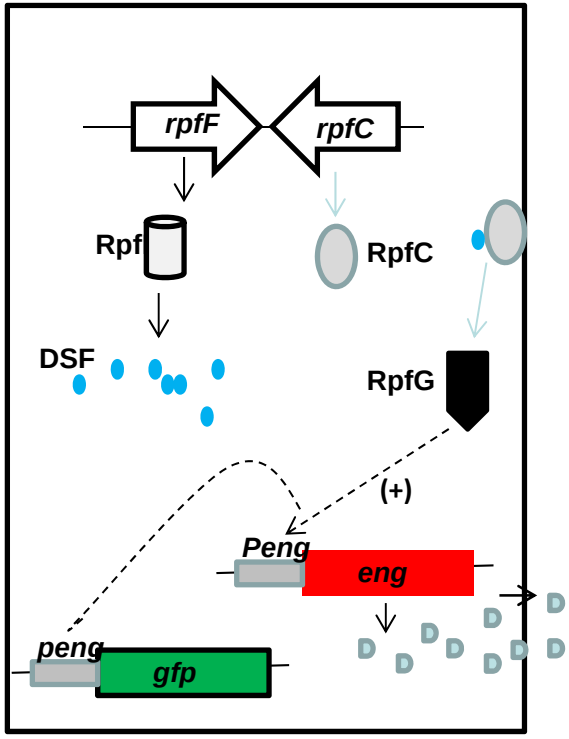
Pseudomonas syringae
pv. *syringae* (*Pss*)

A



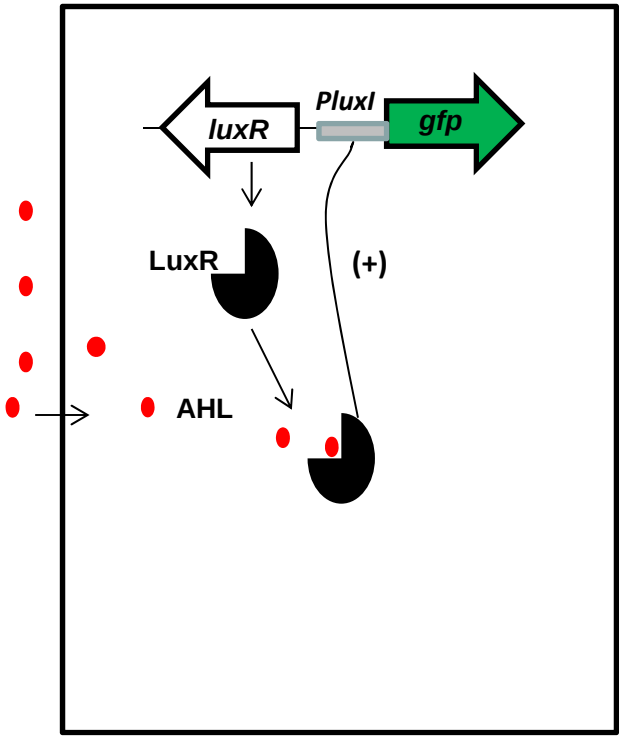
Xanthomonas campestris
pv. *campestris* (Xcc)

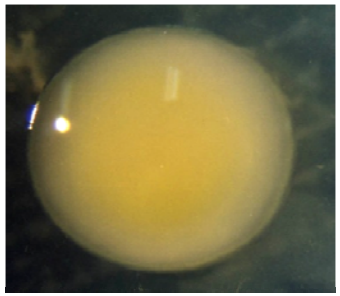
B

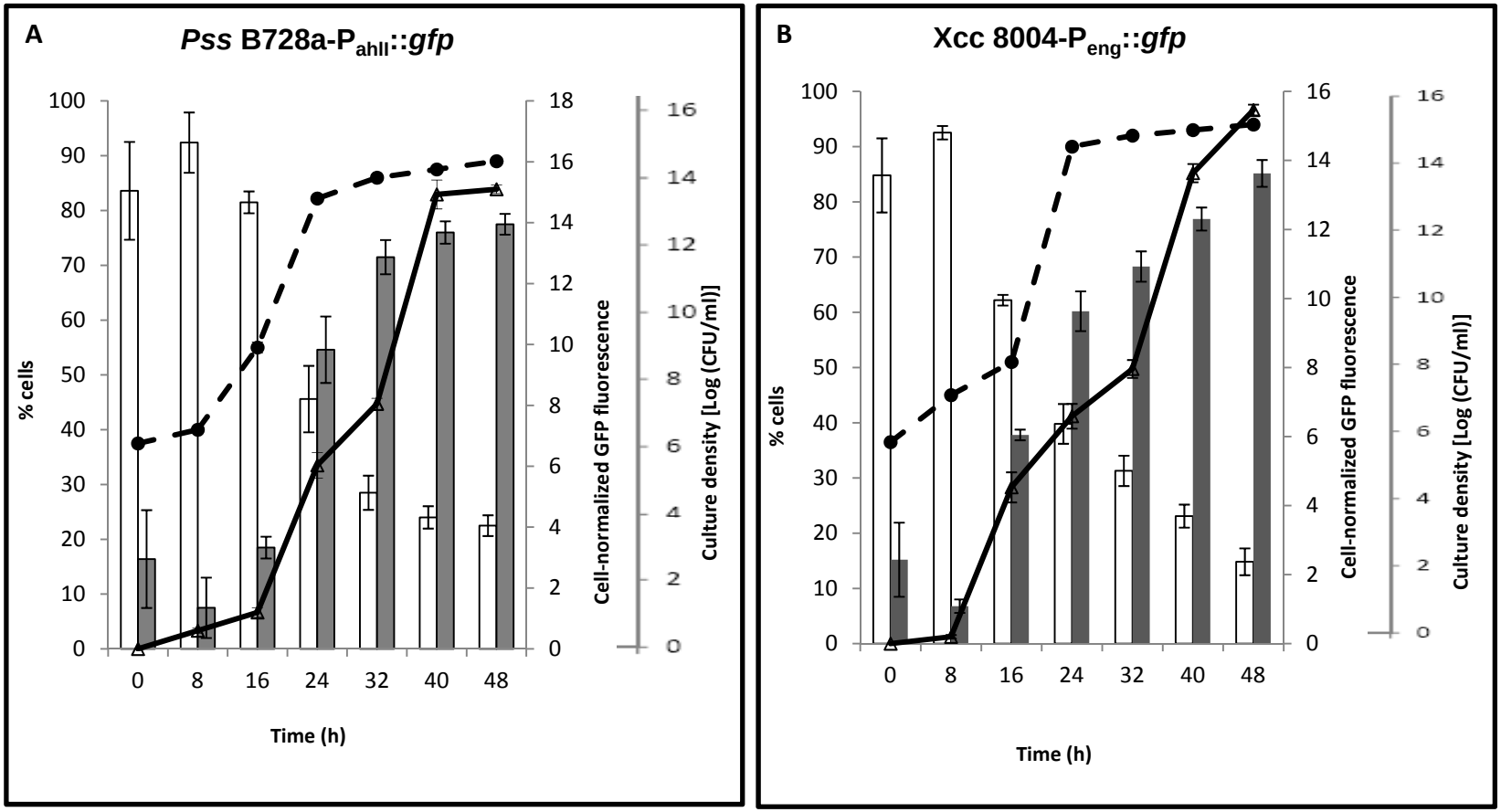


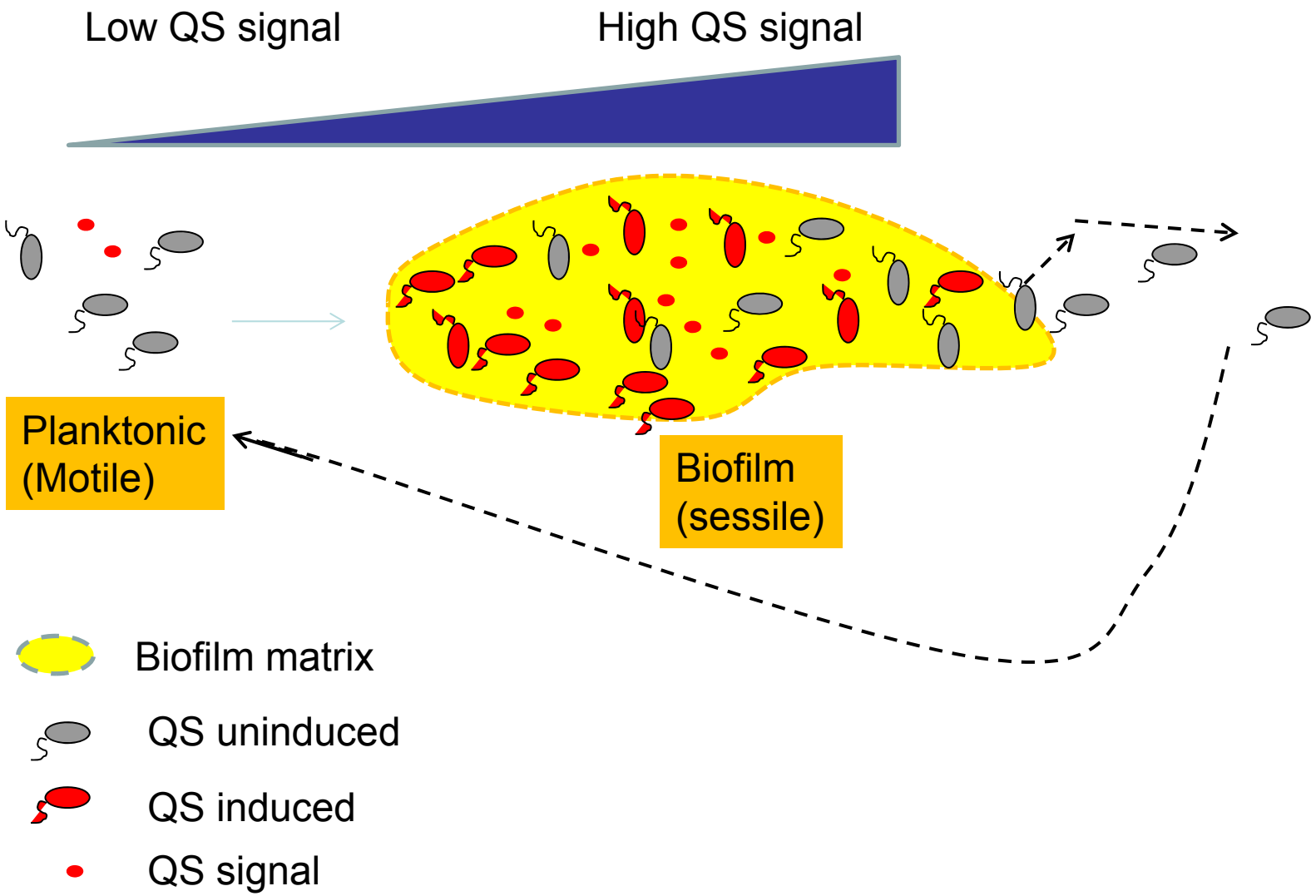
E. coli (JB524)

C






A
Pss B728a-Pahll::gfp
% cells
Cell-normalized GFP fluorescence
Culture density [Log (CFU/ml)]
Time (h)
B
Xcc 8004-Peng::gfp
% cells
Cell-normalized GFP fluorescence
Culture density [Log (CFU/ml)]
Time (h)



***Molecular Microbiology*; (2014)**92:557-569

MEDICINE

A Weakness in Bacteria's Fortress

Evolutionary biologists are trying to attack bacteria in a new way: by short-circuiting their social life

By Carl Zimmer

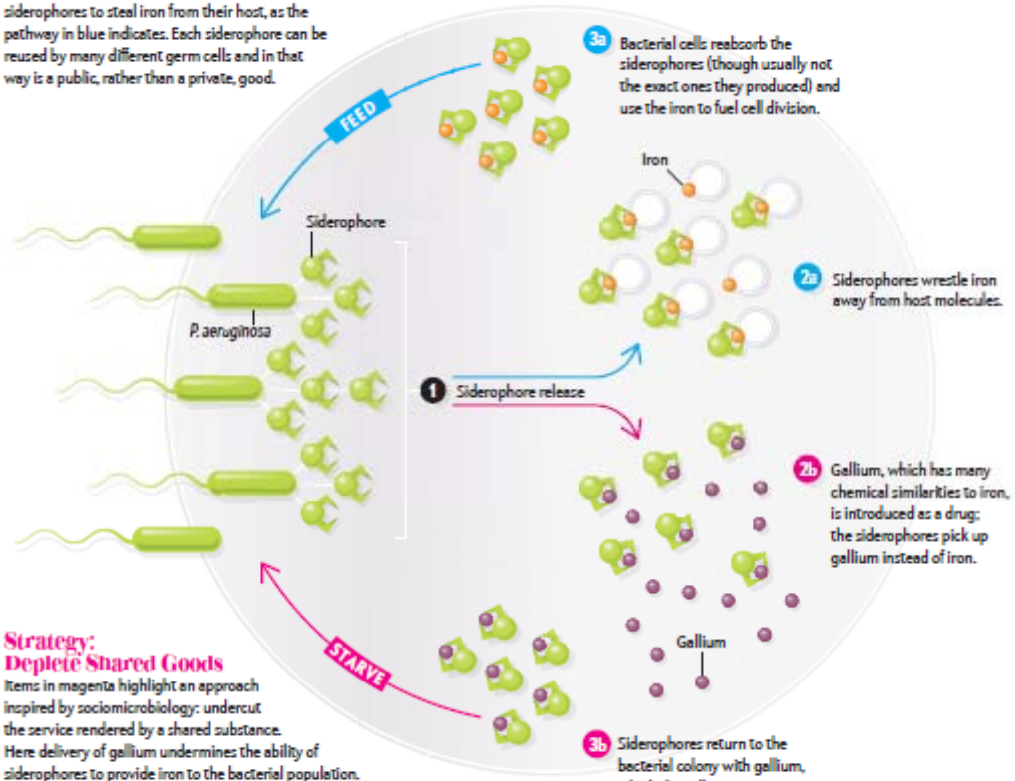
AT THE UNIVERSITY OF ZURICH, ROLF KÜMMERLI INVESTIGATES NEW DRUGS TO STOP DEADLY infections. He spends his days in a laboratory stocked with petri dishes and flasks of bacteria—exactly the place where you would expect him to do that sort of work. But Kümmerli took an odd path to get to that lab. As a graduate student, he spent years hiking through the Swiss Alps to study the social life of ants. Only after he earned a Ph.D. in evolutionary biology did he turn his attention to microbes.

Lessons from Evolutionary Biology

Researchers hope to develop more effective antibacterial treatments by interfering with the way various germs communicate and cooperate with one another. Such an approach should trigger less drug resistance, in theory, because no single cell should be able profit by changing the way it responds. One idea, which targets a molecule that Pseudomonas bacteria use to scavenge iron, is shown below.

The Target: Communal Nutrient Gathering

Pseudomonas bacteria produce molecules called siderophores to steal iron from their host, as the pathway in blue indicates. Each siderophore can be reused by many different germ cells and in that way is a public, rather than a private, good.

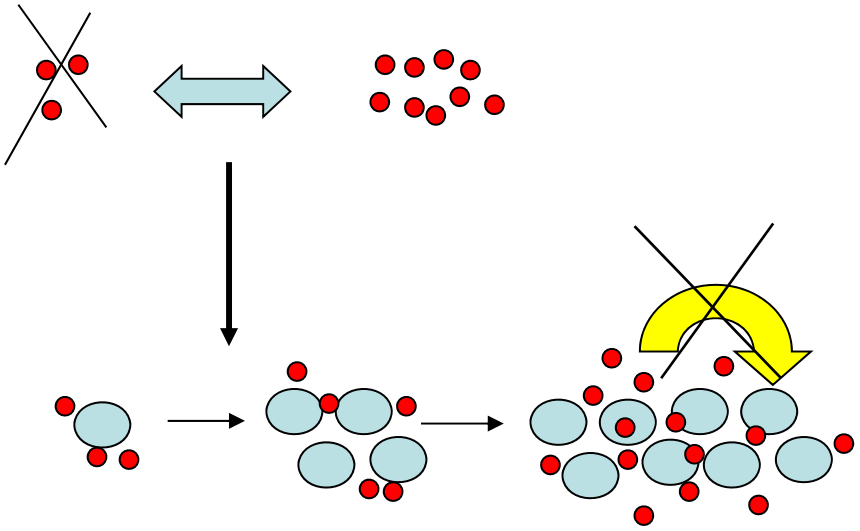


Strategy: Deplete Shared Goods

Items in magenta highlight an approach inspired by sociomicrobiology: undercut the service rendered by a shared substance. Here delivery of gallium undermines the ability of siderophores to provide iron to the bacterial population. Even if a single cell develops a favorable siderophore mutation, that cell will still probably starve because it is likely to take up siderophores made by other bacteria.

Strategies to interfere cell cell signaling in plant pathogen

- ❖Degradation of signaling molecule
- ❖Overproduction of signaling molecule
- ❖ Altering the signaling molecule for causing pathogen confusion

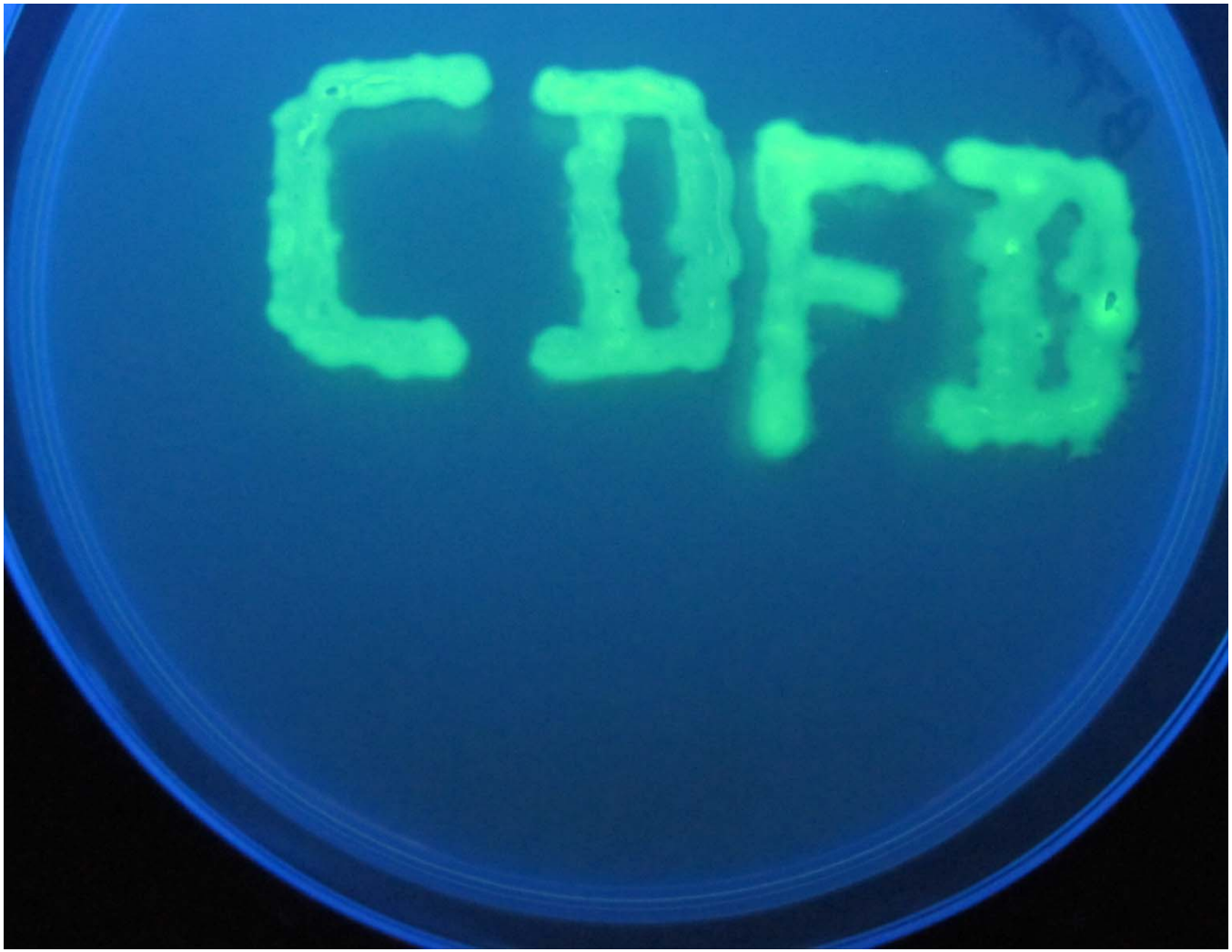


Management of Disease by cell cell signaling interference

Transgenic
Non-transgenic

Social drugs as anti-microbial agent

Quorum Sensing system in bacteria




CDFD
CENTRE
FOR DNA
FINGERPRINTING
AND

UNIVERSITY of BRADFORD

School of Chemistry & Forensic Sciences

Chemistry@Bradford:
A World of Possibilities

An update on the response of functional polymers to cell based targets

Stephen Rimmer

School of Chemistry and Forensic Sciences
University of Bradford, UK

University of Bradford

Premier Technology University

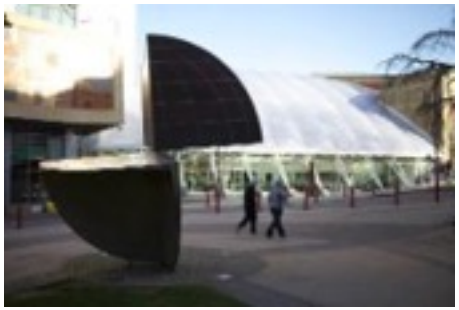
Polymer Science and Engineering

-9 Faculty -Summer 2016
-Good facilities for Polymers

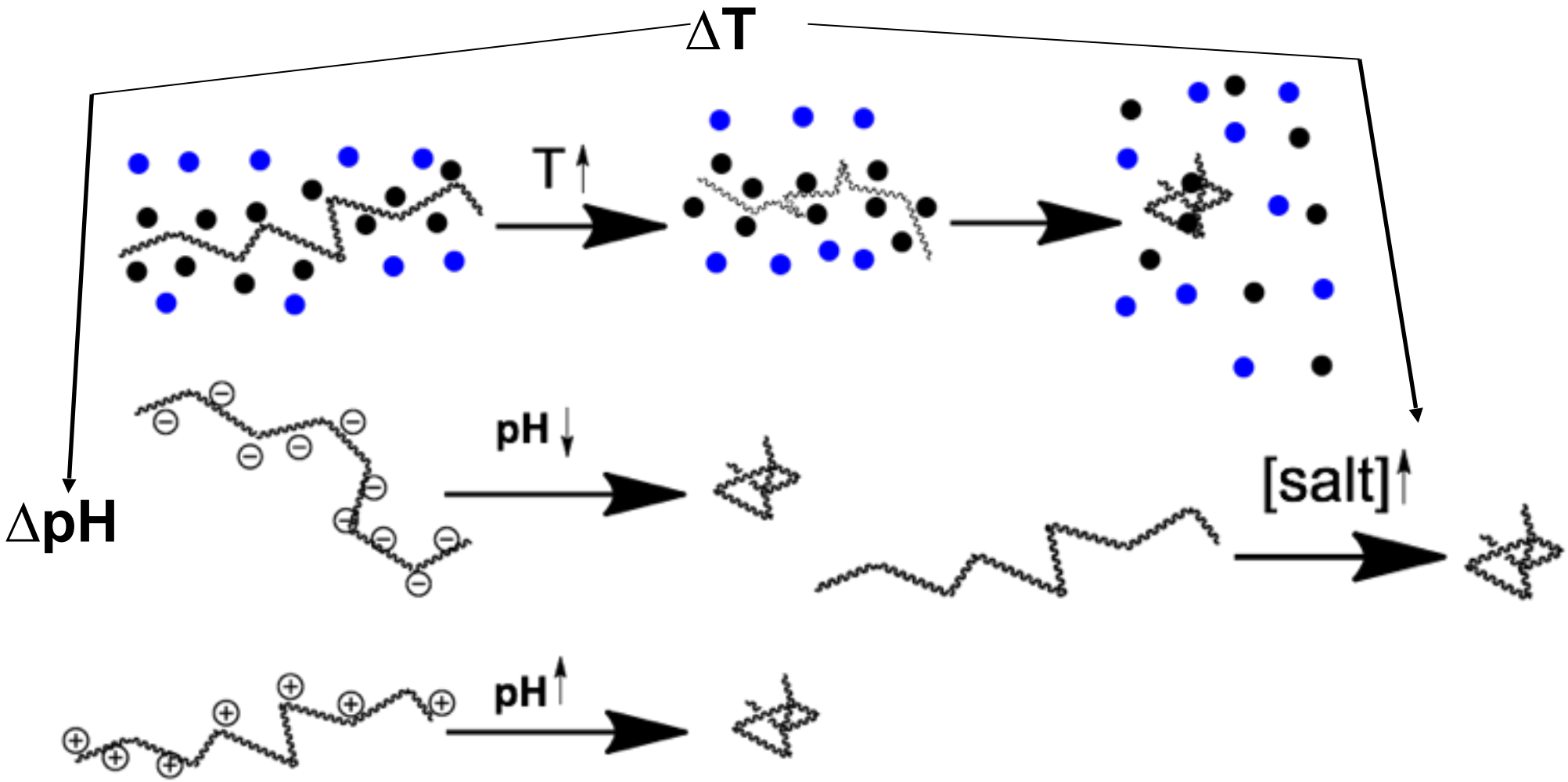
Victorian Industrial city

Based on textiles

Now regenerating



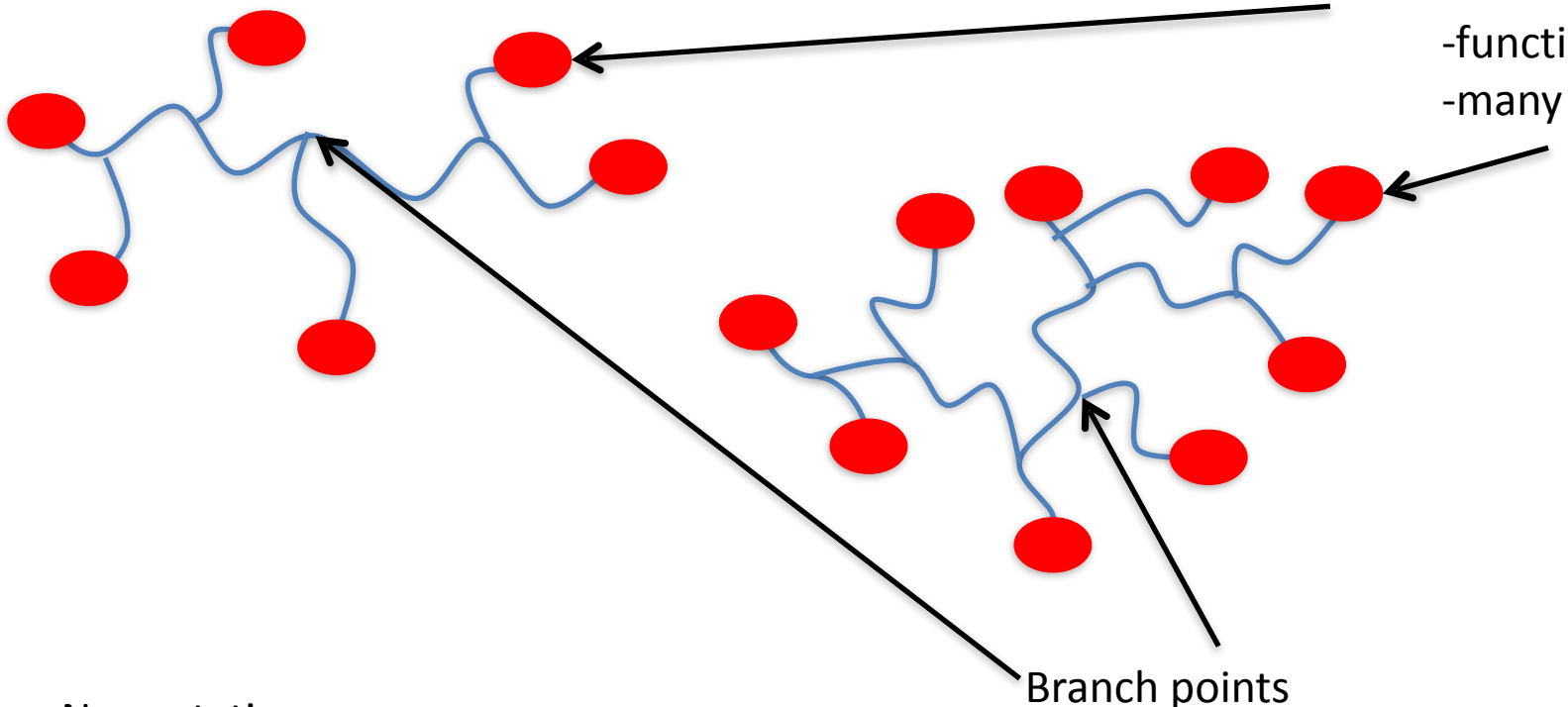
Smart polymers and stimuli



Can we mimic biological "smartness"

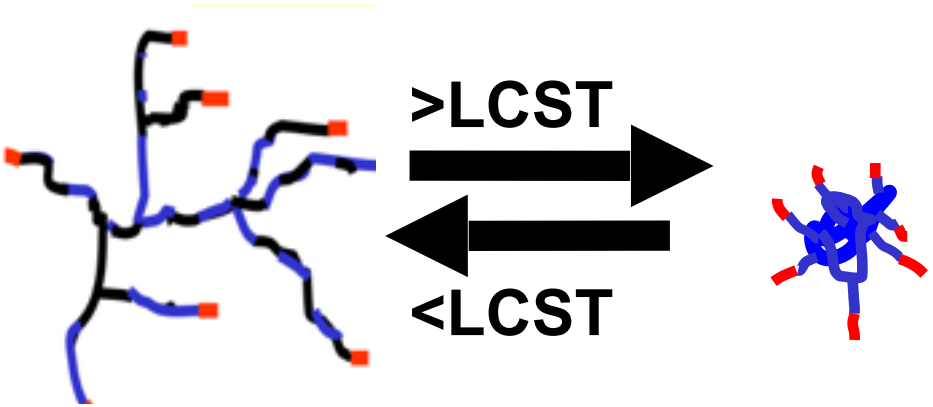
binding ⇨ conformational (folding) change

Some features of branched polymers



No reptation
No or very little inter-segment penetration
Low viscosity
Useful for carrying functionality-as additives

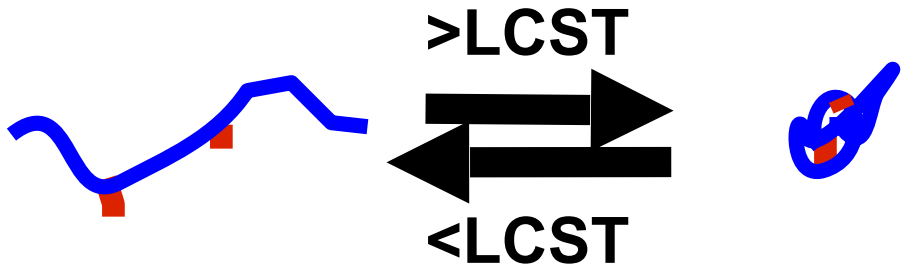
Some consequences of branching: chain ends do not penetrate the coil



Chain ends

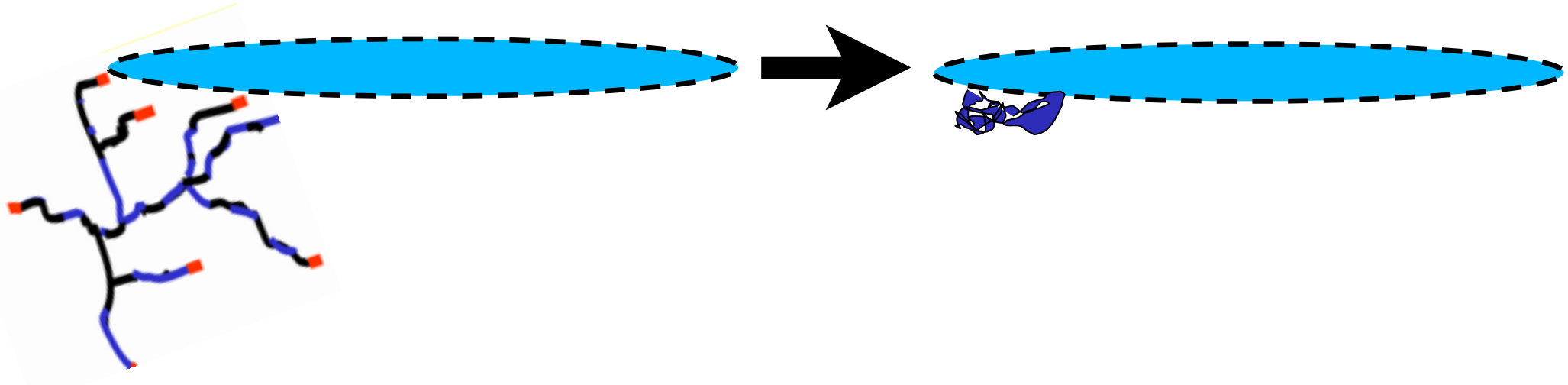
expressed in outer domains

available for binding to cells

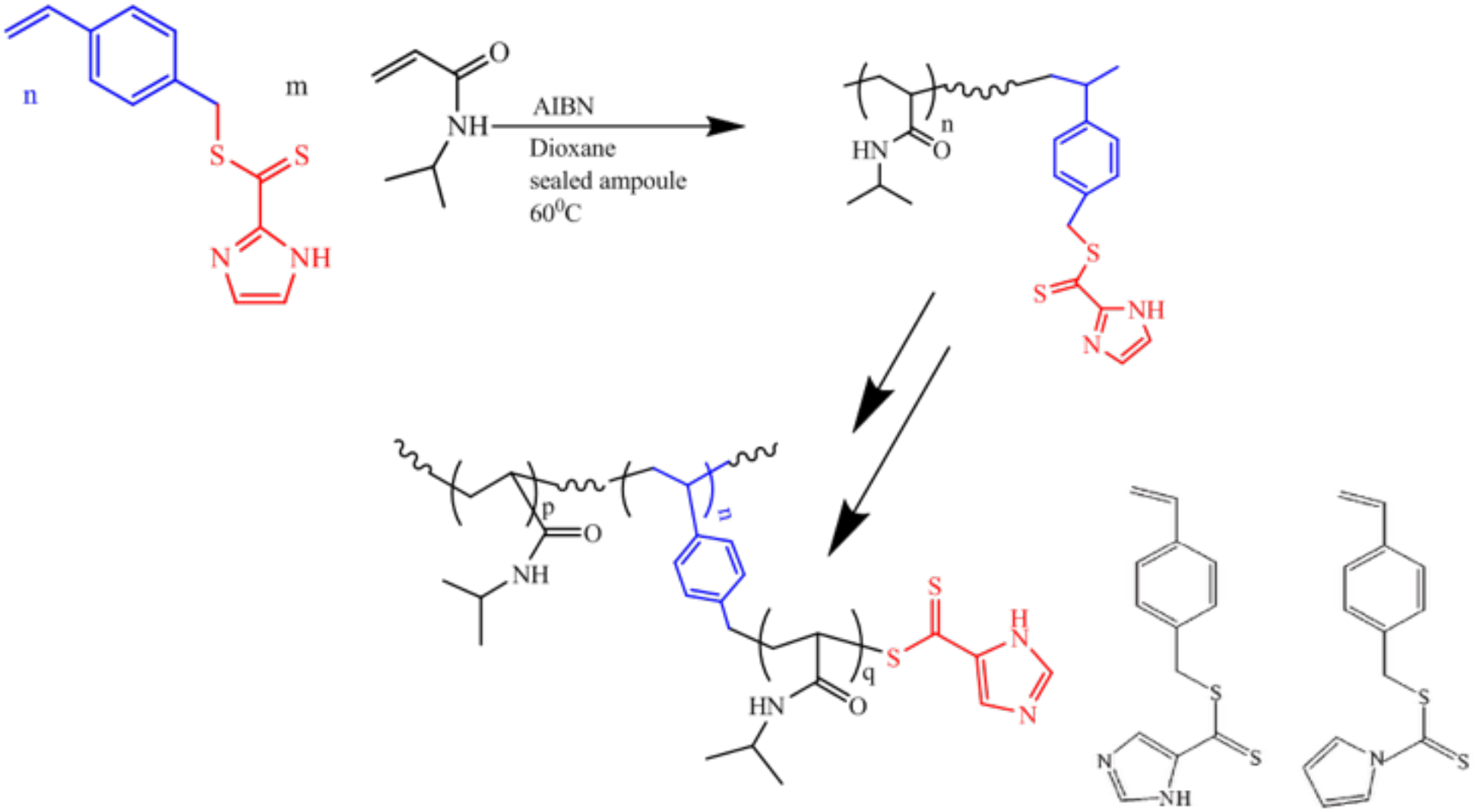


shielded in inner domains

not available for binding

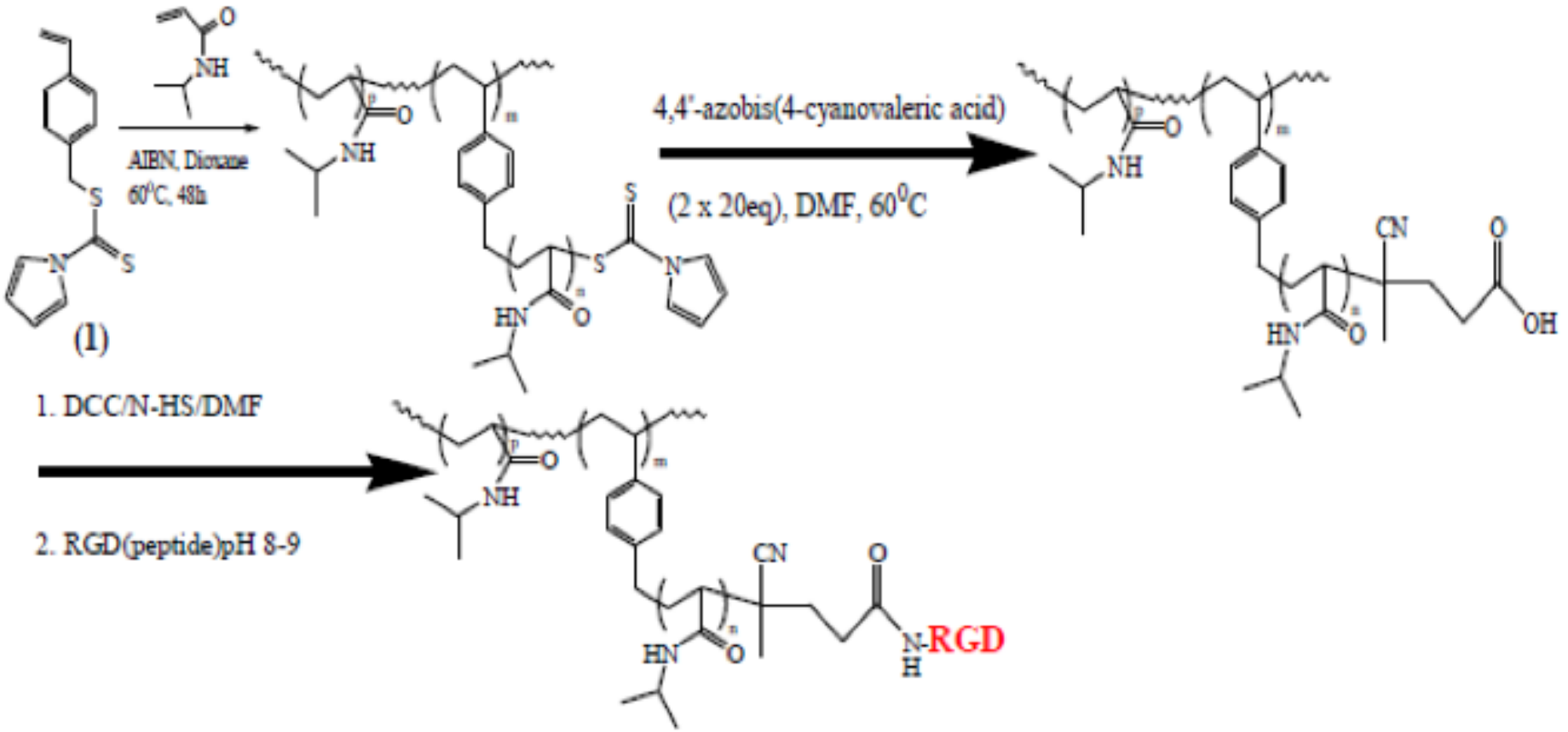


Self-condensing RAFT copolymerisation



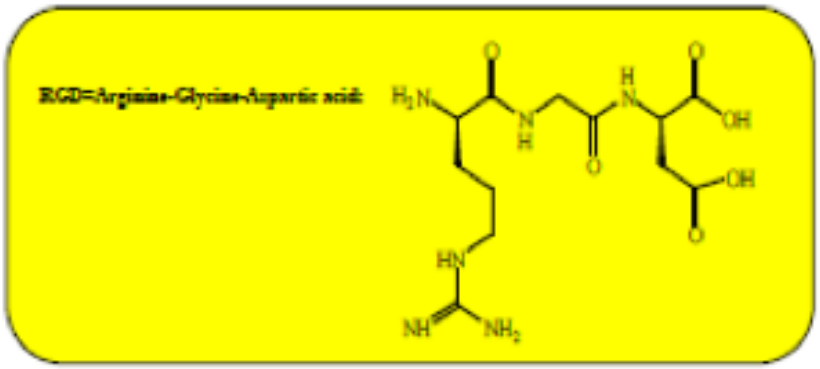
Carter et al *Macromolecules* **38,** 4595, (2005)

Peptide highly branched PNIPAM



Rimmer, Carter, Rutkaite, Haycock, Swanson

Soft Matter 3 971 (2007)

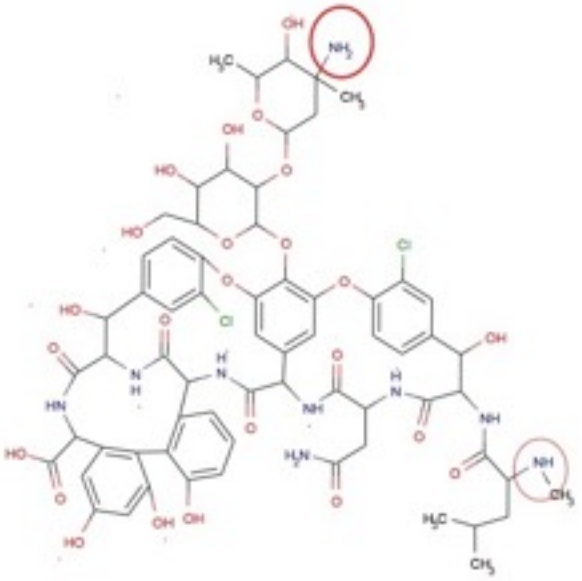


Bacteria-binding ligands

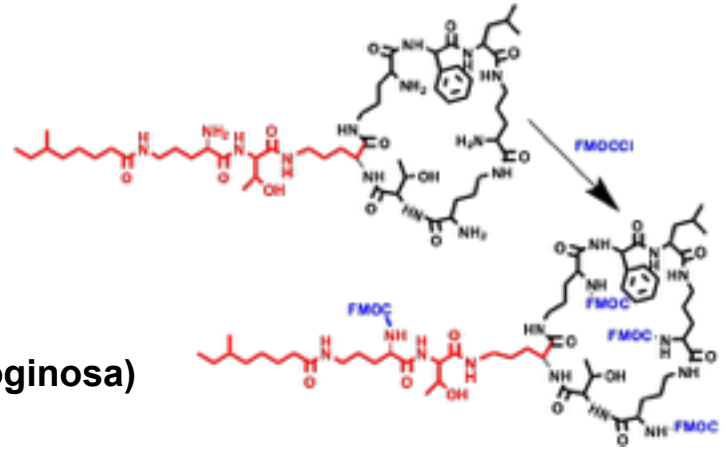
- **Branched poly(NIPAM) - antibiotics @ chain ends**

Vancomycin – binds Gram+ve (*S.aureus*)

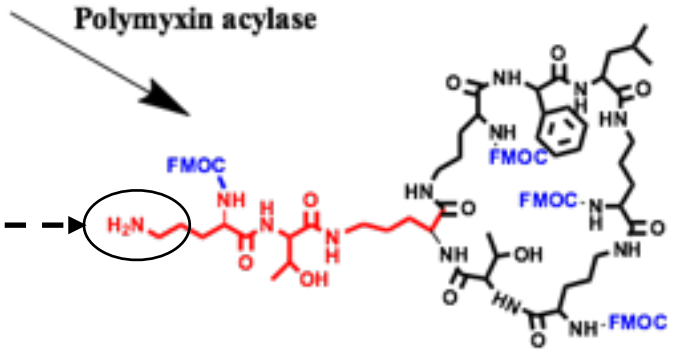
J. Shepherd et al *J. Am. Chem. Soc.*, **132** 1736 (2010)



Polymyxin-binds Gram-ve (P. aeroginosa)

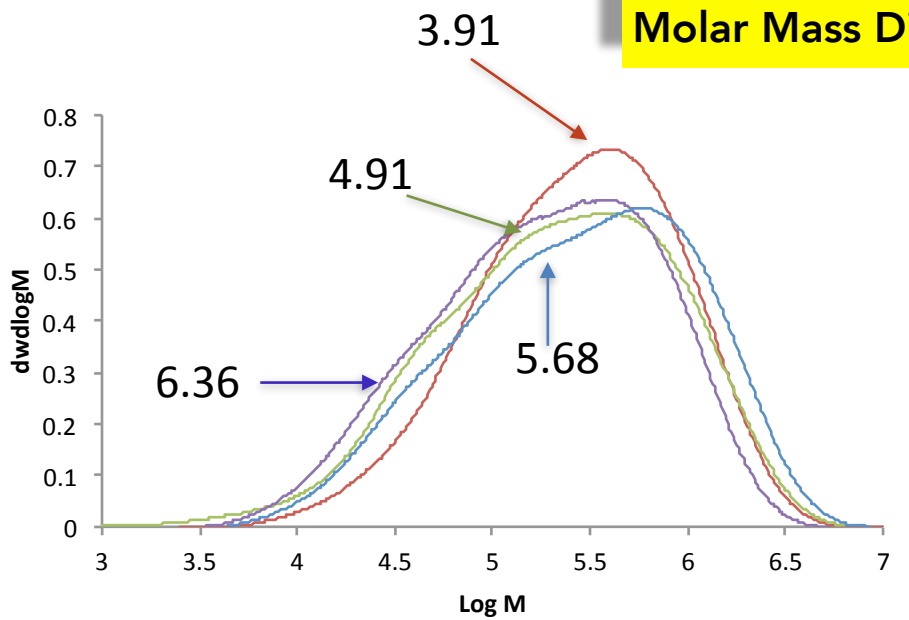


React with COOH on polymer



P. Sarker et al *Biomacromolecules*, **12** 1 (2011)

Molar Mass Distributions

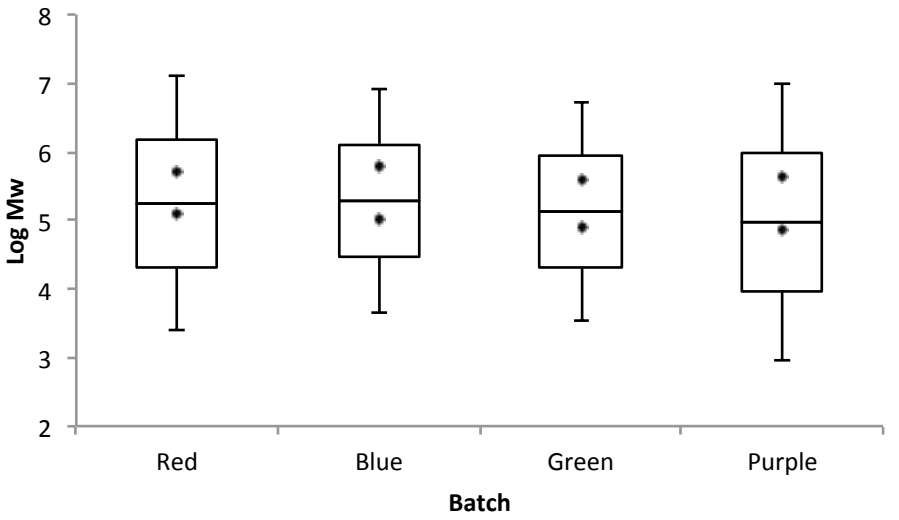


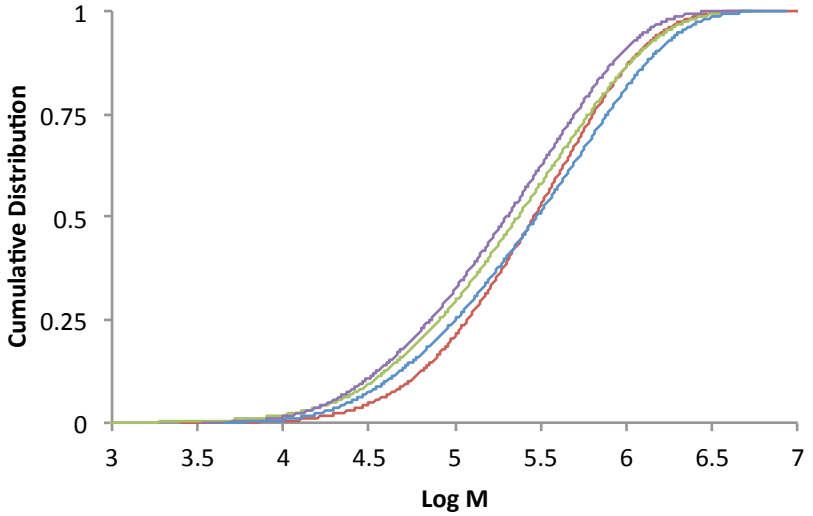
These are broad (bimodal) polymers with a dispersity > 3.

Examining the M_n and M_w is not sufficient to properly explain the distribution…

For example: four repeats

	Red	Blue	Green	Purple
Mn	126184	103643	76823	70031
Mw	493820	588896	377199	445454



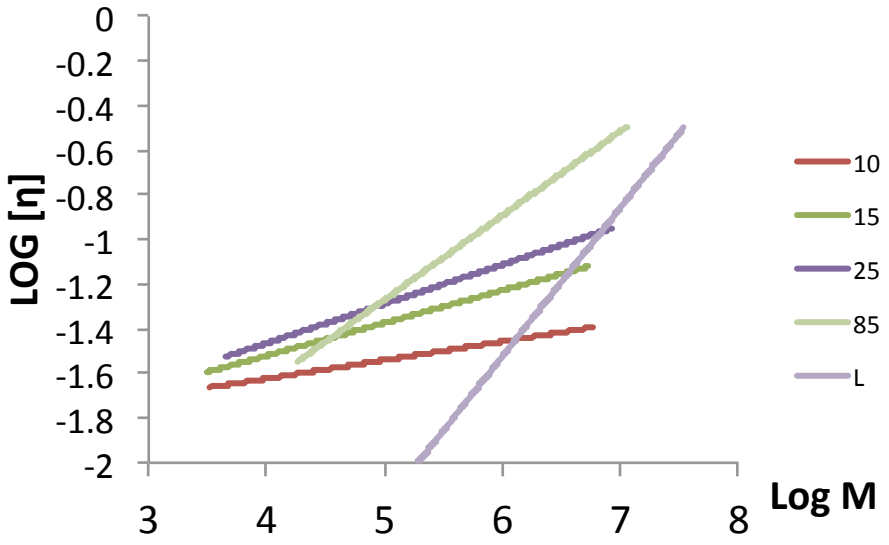


GPC with viscometric detection

$$\text{Log } [\eta] = \text{LogK} + \alpha M$$

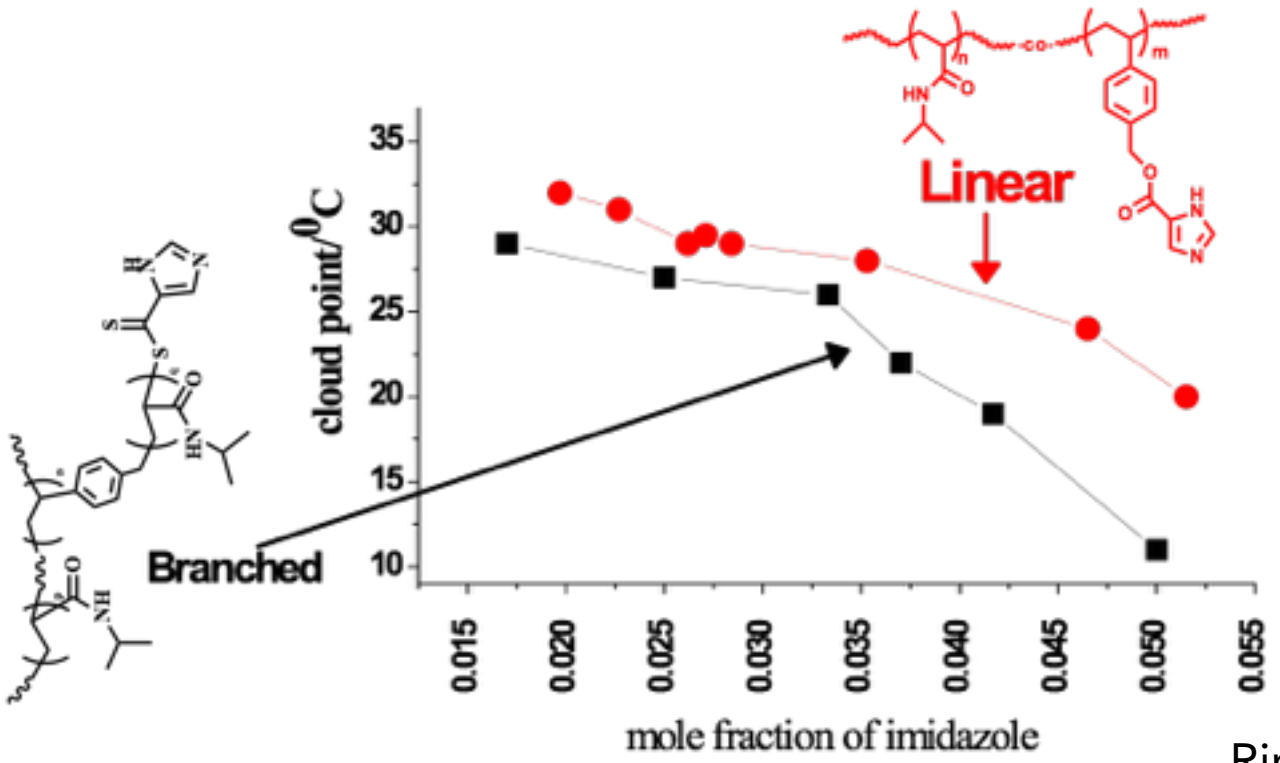
Using the Mark Houwink relationship α tells us about coil shape

α < 0.5 indicates a compact (branched) structure

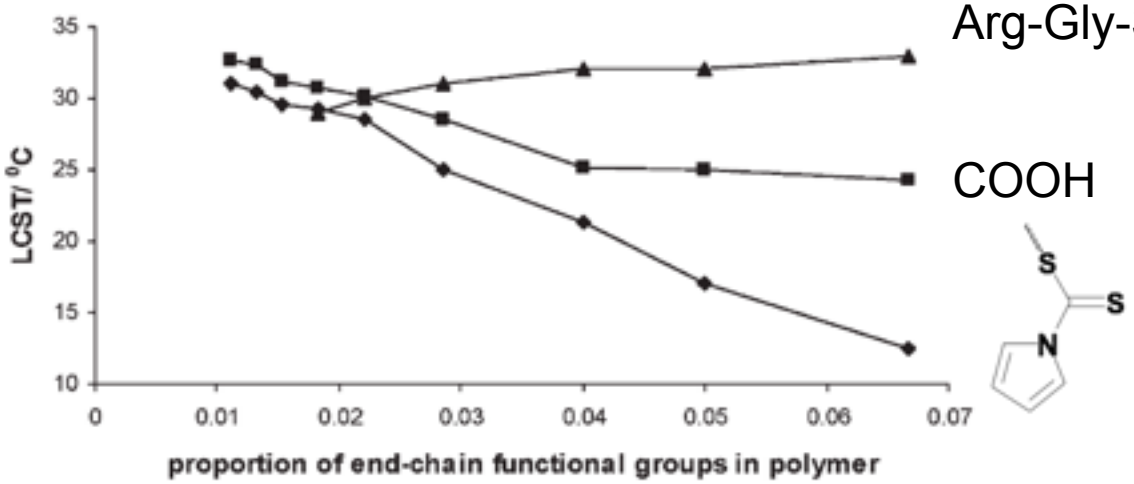


X NIPAM : **1**	Mn	Mw	D	α
10	68469	459510	6.70	0.08
15	74263	452073	6.09	0.15
25	103643	588896	5.68	0.18
85	298724	908581	3.04	0.38
Linear	1298280	2200540	1.69	0.67

Branching and End groups



Rimmer et al *Soft Matter* **3** 971 (2007)



Arg-Gly-Ser

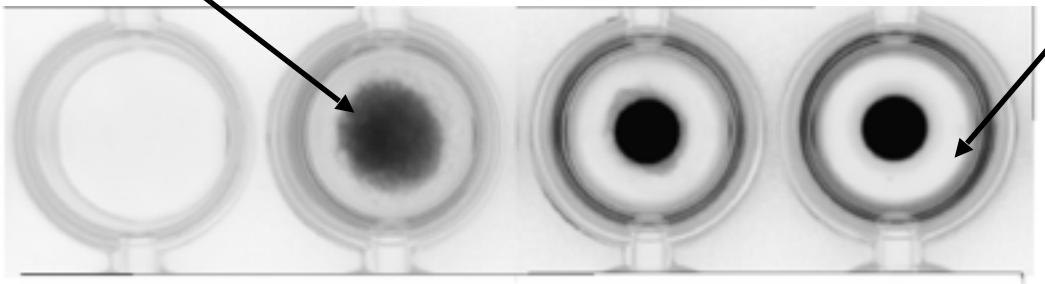
COOH

S. aureus responsive polymers

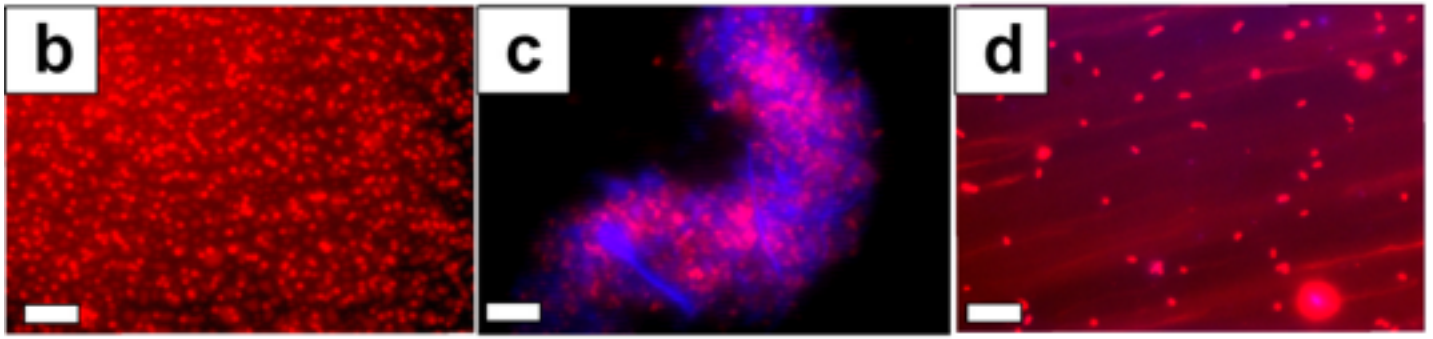
With polymer forms a mat

No polymer forms a button

a

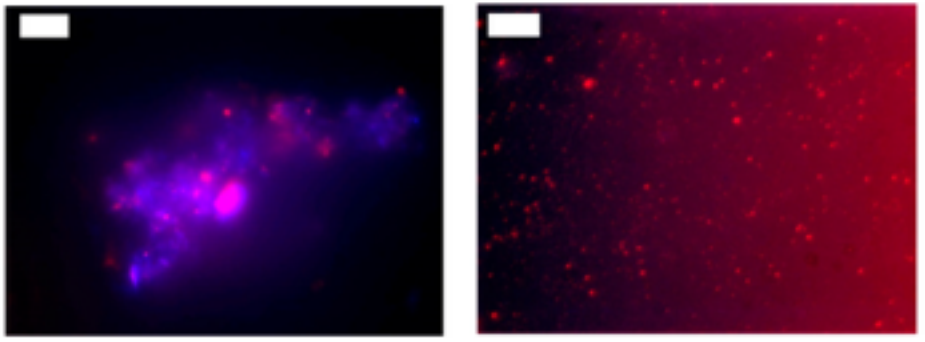


5 +PBS 5 1.25 0 [Polymer] /mg ml^{-1}



S. aureus HB-PNIPAM +*S. aureus* HB-PNIPAM+*P. aeruginosa*

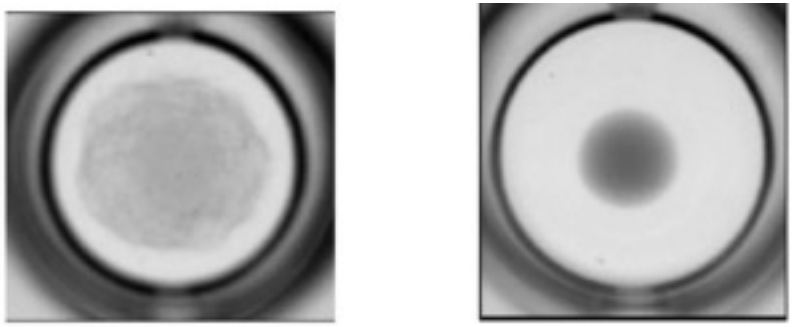
e



37 °C 4 °C

S. aureus

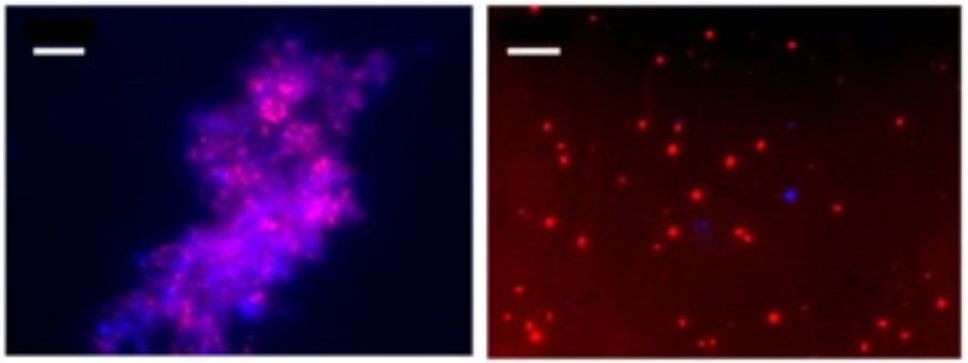
HB-PNIPAM-pmx with P. Aeroginosa: Gram-ve



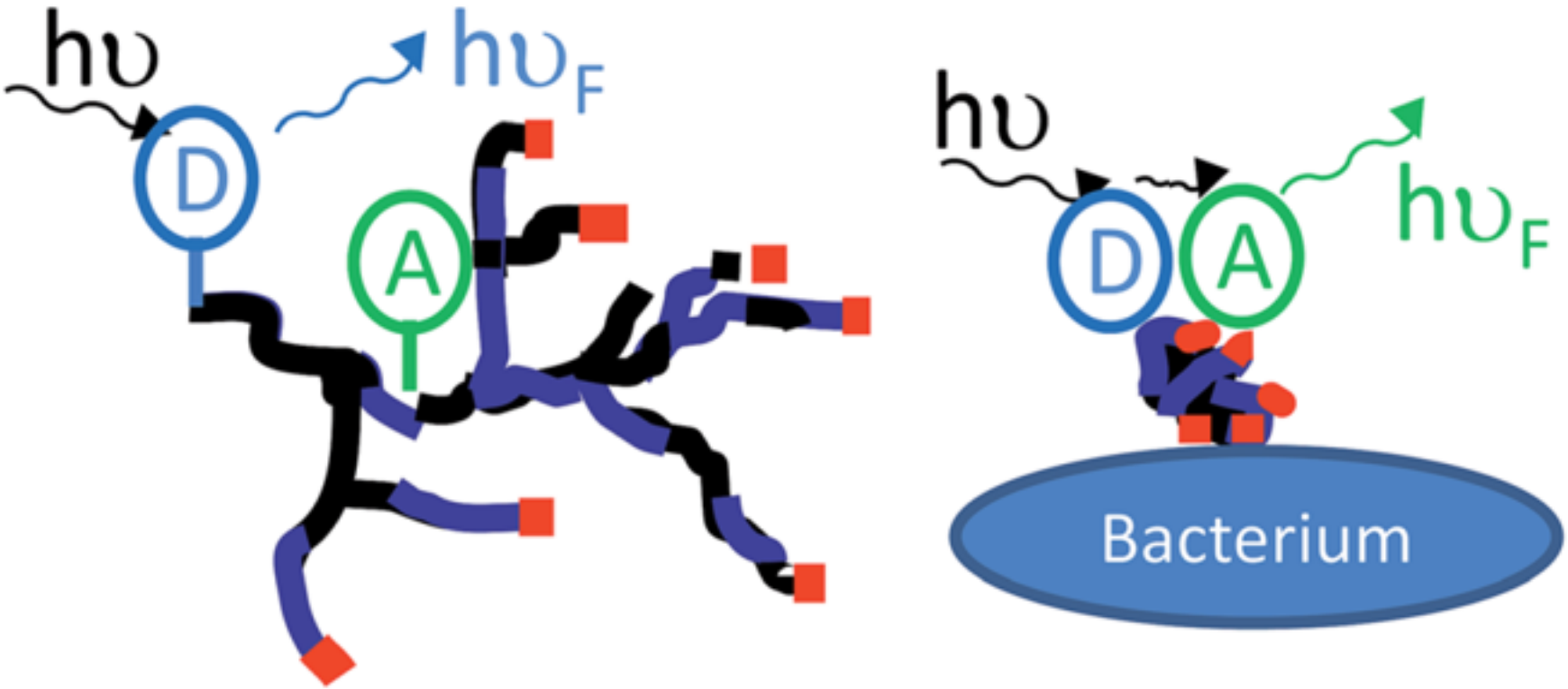
37°C 4°C

37°C / 1h 4°C/18h

HB-PNIPAM-pmx & P.aeruginosa

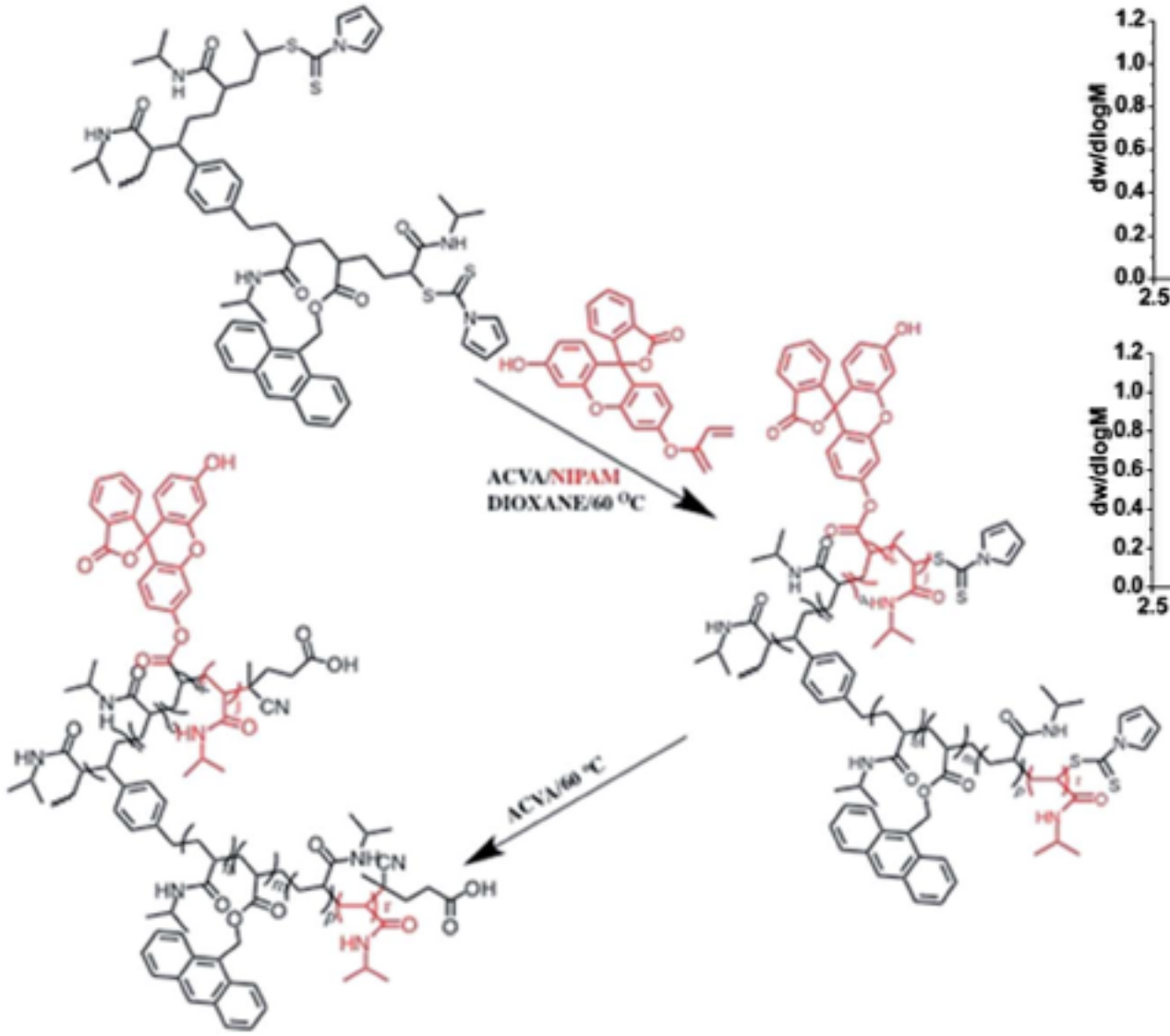


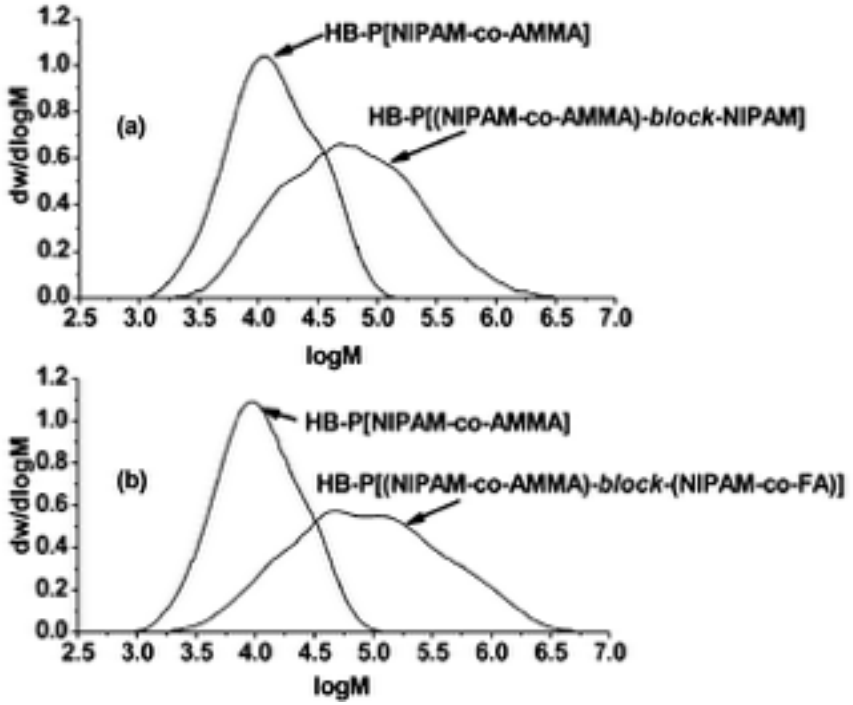
Forster Energy Transfer used to prove binding induced transition



Sarker et al *Soft Matter* **10** 5824 (2014)

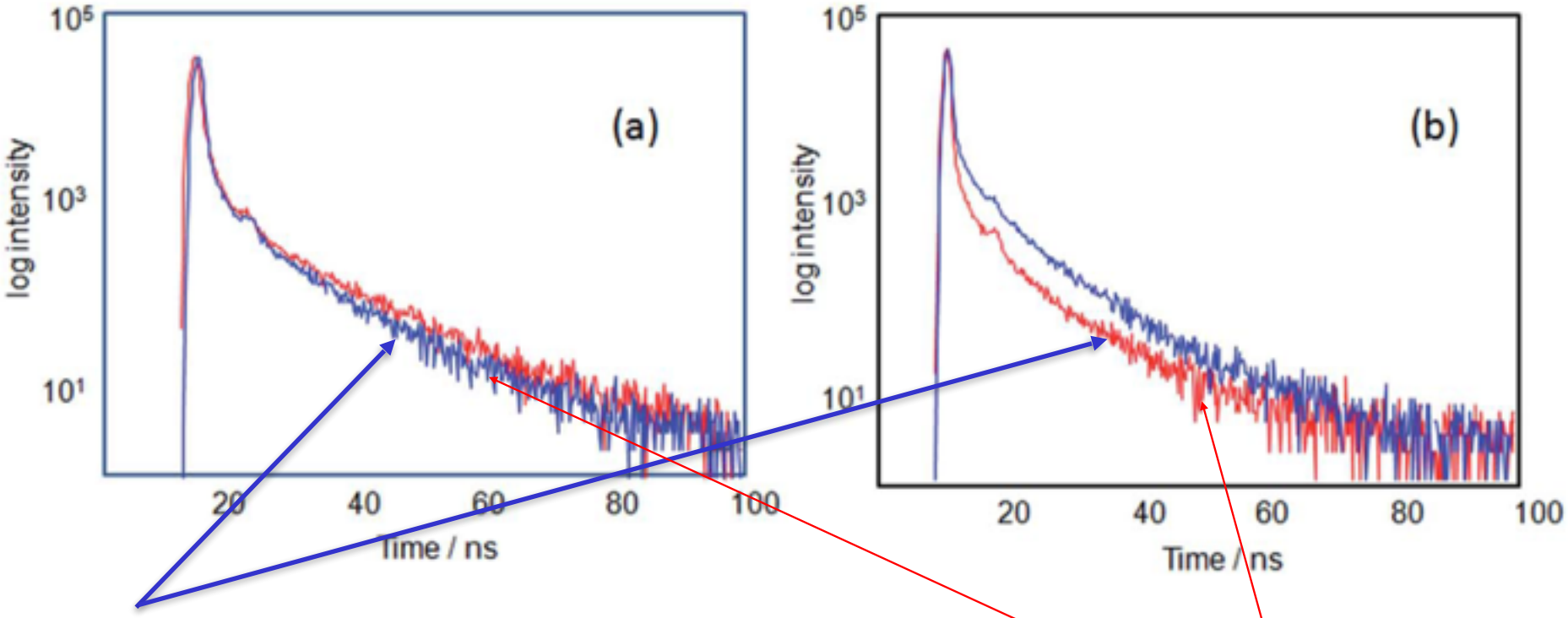
Synthesis of double labelled polymers





Fluorescence decay curves

Water ***S. aureus***



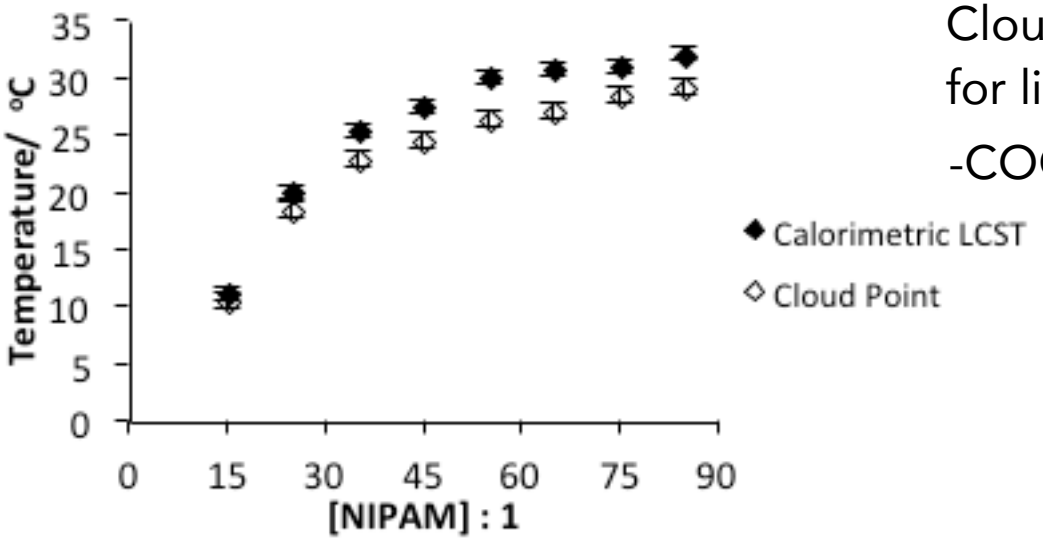
HB-P[(NIPAM-co-AMMA[1%])-block-(NIPAM)]-Van

HB-P[(NIPAM-co-AMMA[1%])-block-(NIPAM-co-**FA**[2%])

Conclusion

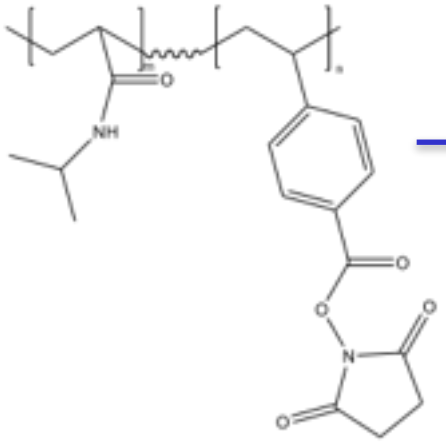
Branched PNIPAM with vancomycin end groups does indeed pass Through the coil-to-globule transition on binding to S.aureus

Linear compared to branched



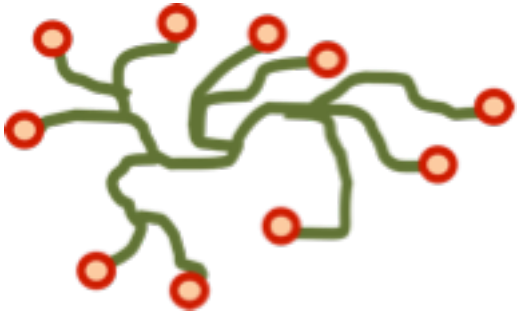
Cloud points and calorimetric LCSTs for linear and branched polymers

-COOH-functional polymers in DI water



vancomycin

L-PNIPAM-Van



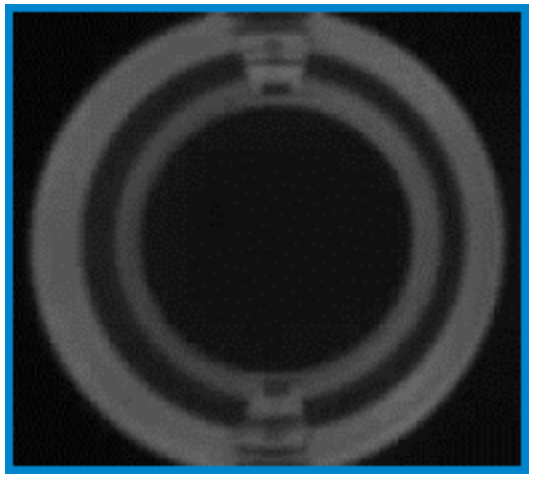
HB-PNIPAM-Van

	Transition temp DSC	Cloud Pt.
L-PNIPAM-Van	35 °C	35 °C
HB-PNIPAM-Van	22 °C	not observed

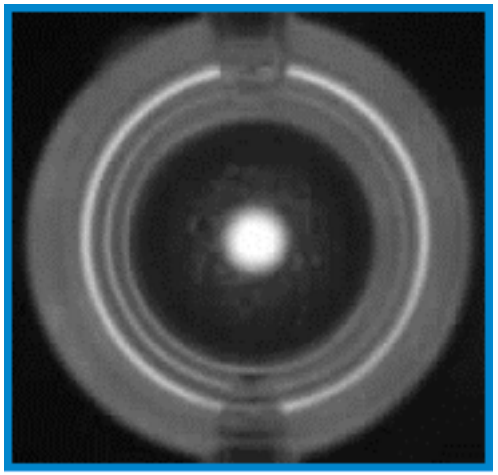
After 24 hours

L-PNIPAM--van incubated

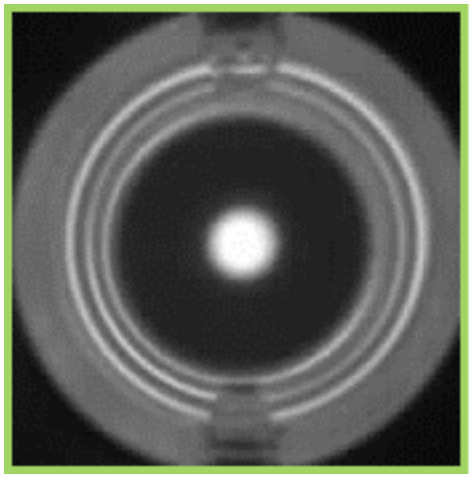
Control L-PNIPAM-van in PBS



with *S.aureus* in PBS



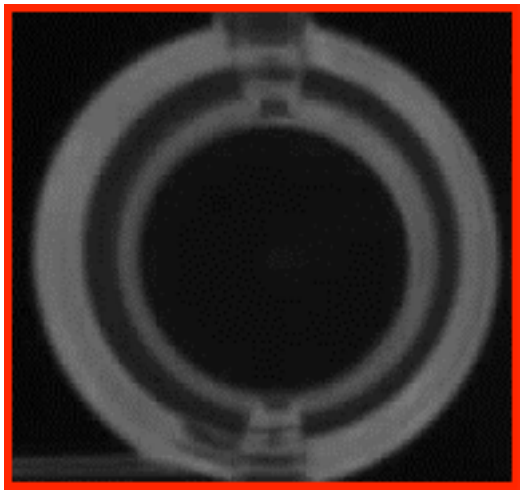
***S.aureus* in PBS**

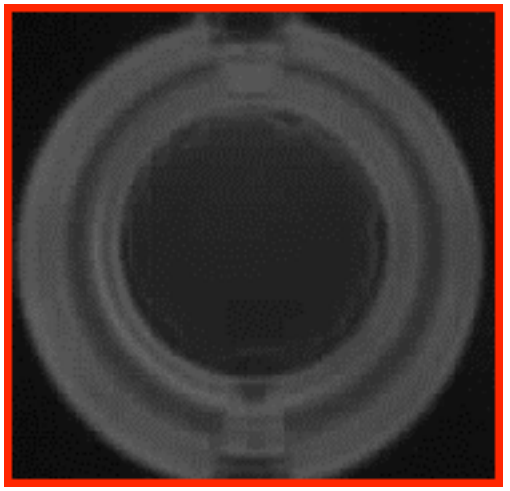


HB-PNIPAM-van incubated

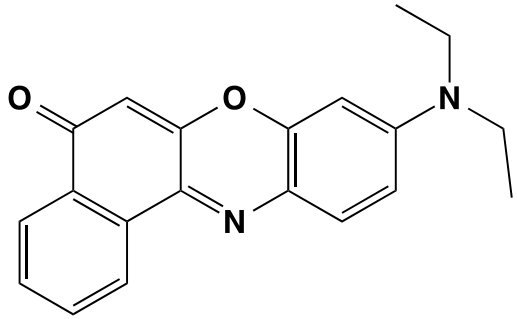
HB-PNIPAM-van in PBS

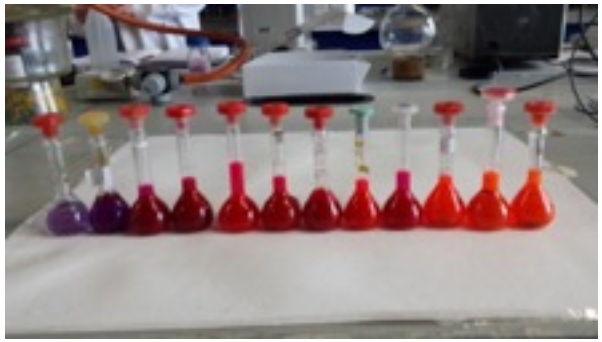
with *S.aureus* in PBS



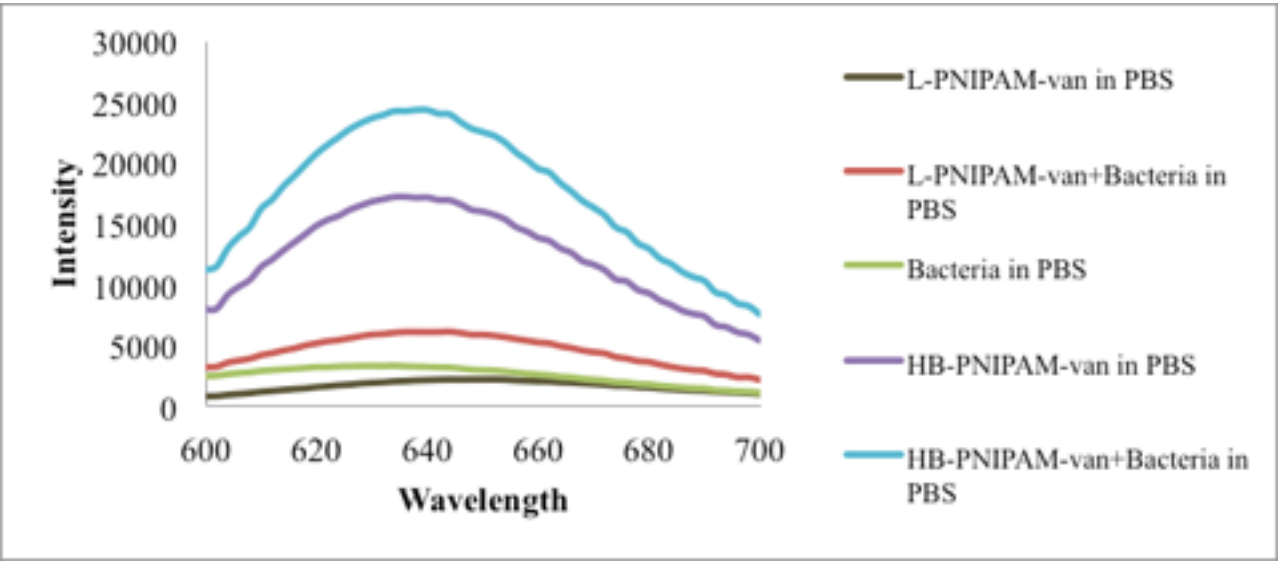


USE OF NILE RED -AS A PROBE





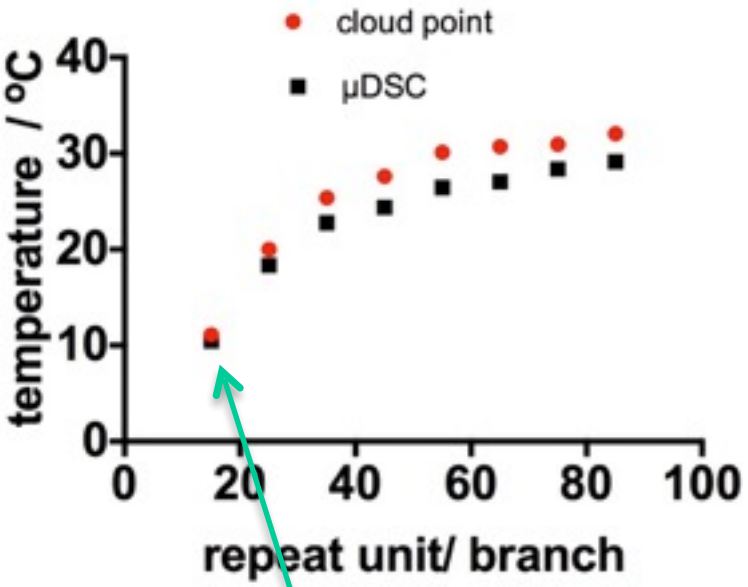
- Solvatochromic dye
- Spectrum changes as polarity changes
- Also fluorescence intensity increases as polarity decreases



- Added to polymer solutions
- plus bacteria
- Only a small shift in λ_{max}
- but fluorescence intensity increases

Behaviour of Nile Red probe as branching increases

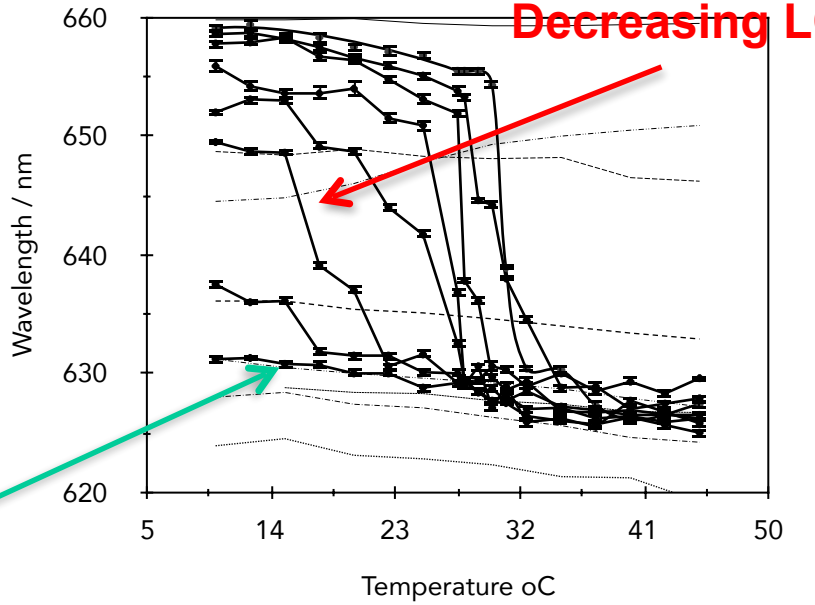
Further look at Nile Red in a range of polymers with COOH end groups and changing degrees of branching



LCSTs decrease as degree of branching increases.

However, using the Nile Red λ_{max}:

But a LCST does occur



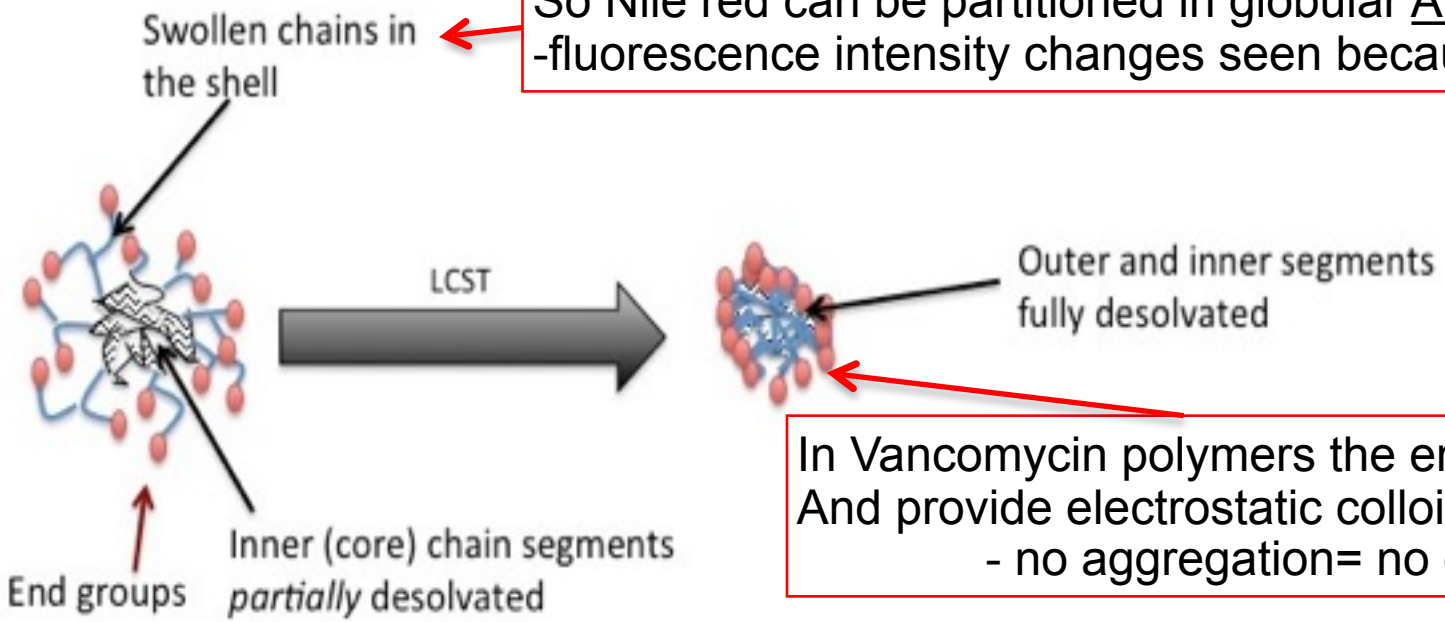
No change at high degrees of branching

An improved model

- λ_{max} does not change much for Nile red in polymers of high degrees of branching

- Fluorescence intensity is enhanced
- With Vancomycin we do not see a cloud point but we do see a LCST in μDSC
- FRET shows that coil-to-globule transition does occur on binding

So Nile red can be partitioned in globular AND open chain domains
-fluorescence intensity changes seen because this is more sensitive



In Vancomycin polymers the end groups are charged
And provide electrostatic colloidal stability
- no aggregation= no cloud point

Polar end groups "penetrate the shell"

Acknowledgements

Richard England
Sally Hopkins
Joey Shepherd
Prodip Sarker
Steve Carter
Kathryn Swindells
Pavintorn Tetaratanatorn
Richard Hoskins
Thomas Swift

Ian Douglas
Sheila MacNeil
Linda Swanson

EPSRC, BBSRC, MOD,
TSB, Wellcome, Industry

Discusssion sessions

Antimicrobial resistance is an important area for international collaboration. As well as reporting our work and introducing this multi-discipinary group to various aspects from different fields we engaged in several discussions.

The outputs from these discussions were two areas that the group felt deserved much greater attention and could form large programmes with great impact in the field. The following summarises these two identified themes, which we intend to work on further over the next 12 months.

Theme 1. Sustained and targeted drug delivery

Stephen Rimmer and Vamsi Krishna Venuganti

1.Background

1.1 *Delivery of antimicrobial agents will be critical to combating resistance and to optimum use of both current and new drugs.* By considering and optimising drug delivery strategies we can:

- Deliver at high concentrations above the minimum resistance concentration (MRC)
- Target and use topical applications
- Reduce the exposure of non-infected organs to antibiotics
- Minimise toxic and tissue damage effects and facilitate the use of a wider range of compounds
- Consider the effective use of macromolecular drugs-e.g. antimicrobial peptides

1.2 *Current delivery strategies in this area appear to be under developed and systemic delivery of antibiotics is the norm.* This brings issues such as the well-known onset of Candida infections and the deleterious effects on gut micro biota.

Many current strategies are aimed at slow release but this is not appropriate for antimicrobial delivery, which requires sustained high local concentrations of antibiotic.

Many systems for targeted delivery have not worked *in vivo*: problems with non-specific interactions; masking of ligands and poor stability of complex structures (e.g. unsuitable *in vivo* stability of vesicles etc.).

High drug loadings are key to this area and this will require new systems because many of the current (e.g. amphiphilic block copolymer vesicles) cannot support the required concentrations. This will almost certainly limit the useful systems to solid or lamellar morphologies; either in particle, fibre or membrane form.

2 Aims of delivery systems

2.1 *The delivery requirements for antimicrobials are quite different to other drugs.* Key features are:

- Targeting to bacteria avoiding tissue. This will allow for the use of drugs that might otherwise damage tissue if delivered systemically.
- Rapid release locally
- High concentration sustained delivery
- Local delivery in preference to systemic delivery
- Delivery over 24 hours without redosing

2.2 *Some requirements for multi-drug delivery*. High local delivery may in some cases be required with multiple drugs; e.g. antibacterial and anti-fungal

3. Opportunities from workshop 1, Hyderabad, 14th-15th March 2016

3.1 *Ionphoresis* can be used to progress charged particles through tissue. This is a very useful technology and requires only charged particles to be effective. Charge degradable polymer particles can easily be made with dimensions from 50 -1000 nm using techniques routinely used by a number of the chemistry teams within the group. Possible charged systems, which can be optimsed for ionphoresis, include:

- Graft copolymer and block copolymer micelles with and without end group targeting ligands Graft copolymer and block copolymer lamellar, layer by layer and onion structures, with and without end group targeting ligands
- Highly branched, hyper branched and dendrimer systems
- Core shell emulsion particles
- Imprinted polymer colloids

3.2 *Multiple drug delivery* can be achieved by:

- Simple mixing of drugs; however there may be issues with compatibility and mixing
- Using multi-domain particles; e.g. core shell particles

3.3 *Antimicrobial peptides* have been available for some time but issues with non-adoption include cost and lack of systems for delivery. There are issues with toxicity and it is not possible to administer systemically. New solutions include:

- Delivery by complexing with oppositely charged polymers that mask some of the tissue toxicity
- Enhanced targeting with charged particulate hydrogels
- Using shorter key sequences

3.4 *Targeting* has been an area that received a significant amount of attention but progress into the clinic has been difficult. A significant aspect is that polymer bound ligand affinity is much reduced *in vivo*. Recent results show that by placing the ligands at chain ends, in either branched polymers, block copolymers or low molar mass oligomers, targeting efficiencies are vastly increased. The design of the most successful “linear polymers” for targeting cancer already have ligands at the end of “spacers” but these were designed without the key concepts that we have now developed here. In effect these spacer systems are graft copolymers with the ligands at the chain ends and the principles of their action are the same as the principles at work in the successful targeting using highly branched polymers; i.e. location of chain ends at the outer domains of the polymer coil or globule in branched polymers preventing shielding. Using this concept, drug delivery systems can be targeted to infective sites using:

- Highly branched polymers with ligands at chain ends
- Graft copolymer micelles with ligands at chain ends of the hydrophilic branches (this is similar to the essential use of spacer arms in the current linear polymer targeting systems)

- Block copolymer micelles with ligands at the chain ends of the hydrophilic branches

Theme 2. Designing new diagnostic test that can help physicians start appropriate/targeted treatment

Prashant Grag and Ian Douglas

Classically the identification of microorganisms involves the collection of test samples and subjecting these to detailed microbiology work-up consisting of microscopic examination and inoculation of specimen on various culture media followed by a battery of biochemical tests for the identification of the growth on these media. This approach has several disadvantages:

1. This approach requires a well-equipped and standardized microbiology laboratory. Since most laboratories are located in urban areas physicians in semi-urban and rural areas specially in developing nations do not have access to such laboratories.
2. Ordering such tests involves additional cost. The health system or patients often find it difficult to bear this cost.
3. The identification of microorganisms and antibiotic susceptibility tests take 3-5 days. Untill this time patients are treated empirically.
4. Like any other test microbiology tests have false positive and negative results and the test results need to be interpreted keeping this in consideration. Therefore, a negative microbiology does not rule out infection and a positive test does not always means disease being caused by the identified pathogen.

All of the above factors force physicians to use antibiotics and other antimicrobial agents empirically thereby adding to the problem of drug resistance.
Therefore, one of the approaches to prevent indiscriminate use of antibiotics will be to develop simple to use bedside tests that have high sensitivity and specificity and provide results in the shortest possible time (few hours) and without the need to engage sophisticated laboratory facilities.

The workshop participants considered this as one of the crucial aspects and discussed different approaches for which the group had good knowledge of science and expertise to plan and execute collaborative research projects. The following possibilities were discussed:

- Tests based on the use of bacteria binding functionalized polymers: a significant body of work is already in progress and was presented in part by Prof Rimmer during the workshop. The work is being carried out through a joint Wellcome Trust grant awarded to the University of Sheffield and the University of Bradford in UK and L V Prasad Eye Institute, Hyderabad in India. These responsive polymers are described in a series of publications, in which the group showed that by placing bacteria binding ligands at the chain ends of highly branched poly (N-isopropyl acrylamide) (HB-PNIPAM) it is possible to induce a phase transition in the polymer on binding. The phase transition involves switching the polymer from an open and fully solvated state into a desolvated and more compact state. Also, the globular state produces a less hydrophilic environment than the coil state. This change can be detected by techniques incorporating environment sensitive dyes (solvatochromic dyes).

- Bio-sensers based on specific signals produced by microorganisms: Biosensors are defined as analytical devices incorporating a biological material or a biologically derived material or a biomimetic intimately associated with or integrated within a physicochemical transducer or transducing microsystem, which may be optical, electrochemical, thermometric, piezoelectric, magnetic or micromechanical.
  The technique involves immobilization of biological recognition elements using either of enzymes, antibodies or DNA probes followed by their measurements using optical, electrochemical, thermometric or magnetic techniques. There was a lot of interest in exploring different biological recognition elements for the identification of pathogenic bacteria and the group considered to include this as one of the key elements for developing future collaborative research.
- Tests based on Metabolomics: Dr Ghosh discussed use of metabolomics for the identification of microorganisms. The study of metabolome – the complete set of metabolites produced within an organism – is a reflection of enzymatic pathways and networks encoded within the genome. Additionally, the entire composition of metabolites conveys the interplay of developmental processes and a changing environment over the lifetime of an organism. By studying metabolomics we can not only identify the presence of pathogenic microorganisms but also get information on the global outcome of various factors acting on the cell, as well as an accurate snap shot of the actual physiological state of the organism. The group also considered the cost of the apporach and feasibility of use by general physicians practicing in rural or semi-urban areas.

The discussion concluded with majority vote to explore couple of these approaches. The participants will try to develop collaborative grant proposal incorporating these various approaches.